Population biology and grazing processes of the sea urchin *Centrostephanus tenuispinus* (Clark, 1914) inhabiting coral and macroalgal dominated reefs

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Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

.....

(Gayani N. T. Rathnayaka Mudiyanselage)

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Abstract

Sea urchins play a key role as herbivores and bio-eroders, impacting the organization and structure in many marine benthic habitats. Hall Bank reef (32°2.002´S and 115°42.957´E) off Western Australia is unique having high coral cover, which is unusual for high latitude reefs. Although the high density of *Centrostephanus tenuispinus* is believed to be the reason for the absence of macroalgae, lack of knowledge on the biology and ecology of this species hampers our understanding of the functioning these reefs. The main objective of this study was to evaluate the role of *C. tenuispinus* in structuring and functioning of Hall Bank reef with respect to macroalgae-dominated Minden Reef. Benthic surveys and monthly sample collections were carried out at two sites, to assess temporal variation of substrate cover, urchin density, reproductive patterns, feeding habits and bio-erosion. Higher density (2.94 ± 0.14 m^{-2}) of *C. tenuispinus* with small tests at Hall Bank reef indicated low food availability, while lower densities (0.14 ± 0.01 m^{-2}) of larger urchins at Minden Reef indicated high productivity in the habitat. A clear synchronised annual reproductive cycle was recorded. The gametogenic cycle was initiated by decreasing seawater temperature and day length in March, leading to spawning in winter. Minden Reef urchins had a higher Gonadosomatic Index, coinciding with high food availability. Less seasonal variability in substrate composition influenced the reduced variability in diet at Hall Bank reef while pronounced seasonal variation in the diet of Minden Reef urchins coincided with seasonal changes of substrate cover. Bio-erosion rates were positively correlated with sea water temperature. The annual bio-erosion rate in Hall Bank reef was 1017.69 g $CaCO_3$ m^{-2} y^{-1}. Changes in the population of *Centrostephanus tenuispinus*, being the dominant grazer and bio-eroder in Hall Bank reef, can greatly influence the structure and species composition of the reef, and could lead to ecological phase shifts in this reef system at extremes. The comprehensive knowledge acquired through this study on *Centrostephanus tenuispinus* provides baseline data for the region on this particular species which could be used in monitoring and managing reefs with current trends in climate change.

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Chapter 1 - Literature Review and Introduction

1.1 General Introduction

Sea urchins are well known for their unique morphology, phylogenetic position, economic value as a fisheries resource, and most importantly for their ecological role in shallow benthic ecosystems (Adam et al. 2015; Andrew and MacDiarmid 1999; Byrnes et al. 2013a; Byrnes et al. 2013b; Carpenter 1990; Carreiro-Silva and McClanahan 2001; Dumont et al. 2013; Graham and Nash 2013; Lawrence 2013). As keystone species in many marine benthic habitats they are capable of influencing both the organization and structure of the habitat through herbivory and bio-erosion (Bak 1990; Byrnes et al. 2013b; Carpenter 1990; Coyer et al. 1993; Glynn and Manzello 2015; Lessios et al. 1984b; Mumby 2006; Scheibling 1986). In addition, sea urchins are habitat providers and producers of particulate organic matter to the eco-system (Mamelona and Pelletier 2005; Patton et al. 1985). Thus, presence or absence of sea urchins and their abundance in an ecosystem is crucial for the functioning of many benthic ecosystems.

Being keystone herbivores in many tropical and temperate coral reefs sea urchins are capable of altering diverse nature and function of coral reef systems (Adam et al. 2015; Carpenter 1990; Ling et al. 2010; Scheibling 1986). Ecological shifts to alternative states have been witnessed due to catastrophic mortality of sea urchins in reefs in Caribbean and north Atlantic coasts (Adam et al. 2015; Brady and Scheibling 2005; Hughes 1994; Idjadi et al. 2010; Lauzon-Guay et al. 2008; Scheibling and Lauzon-Guay 2010; Scheibling and Stephenson 1984). Hence, their ability of driving ecological state shifts in the system has gained considerable attention for decades (Adam et al. 2015; Bronstein and Loya 2014).

With the current global trends in human induced environmental degradation, many sea urchin habitats such as coral reefs and rocky reefs are becoming vulnerable to rapid change. The need for species-specific baseline information on biology and ecology on a local scale is essential for effective conservation efforts. This study focussed on filling in knowledge gaps of reproductive biology, population status and feeding ecology of sea urchin *Centrostephanus tenuispinus* in two contrasting reefs of Western Australia.

1.2 Impact of climate change on urchin habitats

Many studies have suggested that rapid change in climate can lead to drastic changes in our oceans (Brierley and Kingsford 2009; Byrne and Przeslawski 2013;

Doney et al. 2011; Dupont et al. 2013; Hoegh-Guldberg and Bruno 2010; Kroeker et al. 2013; Poloczanska et al. 2013). With the increasing rate of human interference, many reef systems have become vulnerable to irreversible changes (Edinger et al. 1998; Nyström et al. 2000). A study carried out over 27 years on the Great Barrier Reef has revealed a reduction in coral cover from 28 % to 13.8 % (0.53 % a^{-1}) (De'ath et al. 2012). Recent frequent bleaching events have led to further reductions of coral cover in many reefs (Abdo et al. 2012; Alemu and Clement 2014; Depczynski et al. 2013; Moore et al. 2012b).

High latitude regions have been found to be more vulnerable to these changes (Cheung et al. 2009; Cheung et al. 2012; Wassmann et al. 2011). These changes impact on the species compositions and abundance and further on inter- and intra-specific interactions (Brown et al. 2014; Doropoulos et al. 2012; Poore et al. 2013). Being a vital component of many reefs, sea urchins are one of the primary candidates to be influenced by such changes in the reef ecosystem. Enhanced global warming and changes in ocean chemistry due to changes in atmospheric carbon have been major concerns of changing reef environments over the last century (Burke et al. 2011; Hönisch et al. 2012; Solomon et al. 2009).

1.2.1 Global warming and latitudinal shifts of species

Latitudinal shifts of marine species are inevitable with elevating sea water temperatures, and many researchers have documented species shifts to high latitudinal regions (Freiwald et al. 2016; Ling et al. 2009b; Parmesan 2006; Perry et al. 2005). Agatsuma and Hoshikawa (2007) recorded the expansion of the geographic range of the sea urchin *Hemicentrotus pulcherrimus* in the Sea of Japan, from off southwestern Hokkaido to northern Hokkaido in response to increase in water temperature. Many other studies have indicated similar shifts from many geographical regions (Beck et al. 2016; Connell and Irving 2008; Johnson et al. 2011; Ling 2008). Enhanced global warming has resulted in the migration of some tropical herbivorous fish to temperate waters, increasing in their abundance. Parrot fish *Nicholsina usta* which recently extended its range to temperate waters is known to consume more seagrass than other native herbivores (Fodrie et al. 2010; Vergés et al. 2014). Sea urchins are capable of readily adapting to new environments (Lau et al. 2009). Shifts in ranges of keystone species such as sea urchins can directly influence the newly colonised habitat and its inhabitants in many ways. *Centrostephanus rodgersii* in Tasmania is known to create barrens in newly established habitat, negatively impacting the abalones and kelp (Johnson et al. 2005). On the other hand, drastic changes in ocean chemistry, leading to

lower pH levels influence many calcareous organisms, which play an important role in all marine ecosystems (Bates et al. 2014; Sabine and Feely 2007; Sabine et al. 2004; Zeebe and Wolf-Gladrow 2001).

1.2.2 Impact of Ocean Acidification

The fifty percent increase in atmospheric carbon dioxide during the last 30 years has led to drastic changes in ocean chemistry as oceans tend to absorb carbon dioxide (Bates et al. 2014; Sabine and Feely 2007; Sabine et al. 2004; Zeebe and Wolf-Gladrow 2001). Decrease in ocean pH (8.2-pre-industrial levels to Current 8.1) has become a major focus of many recent climate change studies (Doney et al. 2011; Doropoulos et al. 2012; Feely et al. 2004; Hendriks et al. 2010; Kroeker et al. 2013; Kurihara et al. 2013; Maier et al. 2009; Melzner et al. 2013; Steinacher et al. 2009; Veron et al. 2009). The calcification process of many calcareous organisms is influenced by the pH of the water. Thus, under low pH; sea water becomes corrosive to calcareous organisms (Doropoulos et al. 2012; Feely et al. 2004; Parker et al. 2013; Stumpp et al. 2012).

Carbon dioxide is highly soluble in cold water, thus cold temperate waters holds more carbon dioxide and become more acidic than warm tropical waters. It is suggested that high latitude surface waters have the potential to be the first to become under-saturated with calcium carbonate among all other marine habitats result in a vast array of changes in different marine ecosystems (Fabry et al. 2008; Pörtner et al. 2005). The level of impact is known to vary depending on taxonomic group and life history stage (Kroeker et al. 2013). At low ocean pH, decrease in the calcification rates (Leclercq et al. 2000; Maier et al. 2009; Wolf-Gladrow et al. 1999), reduction in coralline algae negatively effecting coral recovery, decrease in larval settlement and disruptions in larval behaviour (Doropoulos et al. 2012) slow growth rates of organisms (Doropoulos et al. 2012; Michaelidis et al. 2005), shell dissolution (Shirayama and Thornton 2005), impacts on spawning, changes in early development (Byrne et al. 2009; Clark et al. 2009; Kurihara et al. 2013; Uthicke et al. 2014) and physiological disturbances (Ishimatsu et al. 2004; Pane and Barry 2007; Stumpp et al. 2013; Stumpp et al. 2012) have been documented. Additive effects of low pH and warming can increase the susceptibility of crustose coralline algae to grazing, causing potential cascade effects on other reef organisms (Johnson and Carpenter 2012). Several studies suggest that some species are resilient to ocean acidification over others (Catarino et al. 2012; Schlegel et al. 2012; Sunday et al. 2014). The giant kelp *Macrocystis pyrifera* has shown physiological benefits from warmer and acidic conditions, yet its main grazer, the sea urchin *Strongylocentrotus purpuratus*, is likely to be impacted negatively, with reduced

feeding rates under the same conditions. The impact of climate change on the critical interactions between these two organisms could lead to drastic changes in the ecosystem (Brown et al. 2014).

Recent research suggests ocean acidification can drastically influence both planktonic and benthic marine communities in high latitudes. Ocean acidification coupled with species expansions towards high latitudes can lead to drastic changes in diversity and ecology of high latitude reefs. Being the most affected, temperate reefs have recently gained considerable attention. So far, many temperate reefs have been studied throughout the world; including South Africa (Riegl 2003), USA (Moyer et al. 2003) and Australia (Hatcher and Rimmer 1985; Moore et al. 2012b; Richards et al. 2016; Shears and Babcock 2002; Thomson et al. 2012; Thomson and Frisch 2010).

1.3 Ecology of temperate reefs

1.3.1 Tropical reefs Vs Temperate reefs

Temperate reefs are distinct in many ways compared to low latitude, tropical reefs and possess their own way of functioning (Beger et al. 2014). Physico-chemical factors such as sea water temperature, light intensity and water quality determine not only the presence of a certain species but also their abundance in many reefs. Each reef system is important and unique irrespective of its geographical location. With the main limiting factors such as temperature (18°C-35°C) and light intensity, many coral reefs in high latitudes are in marginal growing conditions (Kleypas et al. 1999; Veron 1995).

In contrast to scleractinian coral-dominated reefs in proximity to the equator, temperate cold water reefs are dominated by macroalgae and soft corals (Edgar, 2001). Phase shifts between macroalgae domination and coral domination can vary temporally and spatially in each reef community, depending on species interactions in reef systems (McCook 1999; McManus et al. 2000; McManus and Polsenberg 2004). Although the majority of previous studies focused on diversity and abundance of tropical reef species, with recent global concerns on climate change, many studies directed more towards reef interactions in temperate reefs (Ban et al. 2014; Kroeker et al. 2013).

1.3.2 Ecological interactions in temperate latitude reefs

Species diversity, abundance of each species, their recruitment and interactions among them has both direct and indirect influences on any reef system. Previous studies have revealed many types of interactions such as symbiosis between zooxanthellae and scleractinian corals, soft coral-hard coral associations, algal associations, other invertebrate associations and interplay between macroalgae and corals (Bulleri et al.

1999; Chong-Seng et al. 2014; Hughes et al. 1987; Lubchenco and Gaines 1981; Mapstone et al. 2007; McManus et al. 2000; McManus and Polsenberg 2004). Among other taxonomic groups, crustose coralline algae play a vital role in strengthening the reef and inducing the settlement of other taxonomic groups such as scleractinians, sea urchins and sponges (O'Leary et al. 2012; Whalan et al. 2012). The presence of multiple interactions in a reef system indicates the dependency of species on each other. Studying the feeding ecology, population biology, and reproduction of key species is essential to understand these interactions.

1.3.3 Keystone species

The concept of keystone species was put forward in 1969 by Robert T Paine with respect to a predator on a rocky shore (Cottee-Jones and Whittaker 2012; Mills et al. 1993; Wagner 2012). A species whose population is "the keystone of the community's structure", whereby "the integrity and stability of the community are determined by its activities and abundance (Paine 1969,92). This concept has been used in many ecosystems when one species has the ability to control the functioning of the entire ecosystem (Lessios et al. 2001; McClanahan 2000; Wagner 2012). Among the best documented examples, some of the keystone species in coral reefs are: the sea urchin, *Diadema antillarum*, in the Caribbean Sea (Adam et al. 2015; Carpenter and Edmunds 2006; Lessios 1995; Myhre and Acevedo-Gutiérrez 2007), damsel fish coral community (Hixon and Brostoff 1983), and *Acanthaster planci* in Japanese coral reefs (Sano et al. 1987).

1.3.4 Drivers of coral-macroalgae phase shifts

Among other organisms, corals and macroalgae are most dominant and reef community structure depends mostly on competition between each other (Barott and Rohwer 2012). Macroalgae grow faster than both corals and coralline algae, yet mostly the growth is controlled by nutrient levels and herbivory. Dynamics of interactions between hard reef building organisms and macroalgae are understudied. Available knowledge on these interactions, are based on experimental data and simple observations.

Mediation of herbivory for macroalgae and coral interplay is well known through many past studies (Adam et al. 2015; Bonaldo and Hay 2014; Burkepile and Hay 2008; Chong-Seng et al. 2014; Coyer et al. 1993; Edmunds and Carpenter 2001; Ferrari et al. 2012; McCook et al. 2001; Michael et al. 2013; Rasher et al. 2012; Thurber et al. 2012). Recent studies have mainly focused on interactions between coral and

macroalgae (Barott et al. 2012; Rasher and Hay 2010). Since macroalgae are mostly influenced by nutrient levels (bottom-up control) and herbivory (top-down control), any major changes in these factors can lead to changes in their abundance. On the other hand, macroalgae are capable of producing chemical substances resulting in the reduction of coral larvae settlement and increasing in coral larval mortality (Barott et al. 2012; Birrell et al. 2008; Rasher and Hay 2010; Webster et al. 2015). Coral larvae are known to avoid chemical cues from degraded reefs and are primarily attracted to healthy reefs (Dixson et al. 2014).

Each reef has its unique mechanisms for operating and controlling the coral-algae interplay whether it is low latitude tropical or high latitude temperate. Indo-Pacific reefs are different from Caribbean reefs in biodiversity, grazing pressure, life history traits, habitat connectivity, coral reproductive traits and species richness (Roff and Mumby 2012). Many reef resilience models are based on studies of Caribbean reefs; however, it is important to consider the differences between the Caribbean and Indo-Pacific reef systems. Indo-Pacific reefs are more likely to have greater resilience than Caribbean reefs due to greater herbivore niche partitioning in the Indo-Pacific (Roff and Mumby 2012). Coral reefs are affected by many biological and physical disturbances, however influence of the same factor may vary temporally and spatially (Hughes and Connell 1999). Generalization of these effects is a difficult task, and therefore it is essential to study interactions, specifically concentrating on the reefs in different regions on an individual basis.

1.3.4.1 Herbivory

Many studies have suggested that herbivory and predation play a key role compared to other interactions (e.g. commensalism) in an ecosystem. Removal of a dominant predator or herbivore causing drastic changes in the ecosystem has been well documented in many geographical locations (Andrew and MacDiarmid 1991; Bak et al. 1984; Byrnes et al. 2013a; Ferrari et al. 2012; Humphries et al. 2014; Mayfield and Branch 2000; Mumby 2006; Pederson and Johnson 2006). Further, competition among herbivores for space and food drives changes in systems (Byrnes et al. 2013b; Lowry and Pearse 1973; Perreault et al. 2014).

The herbivore community in a reef is composed of many taxonomic groups. The majority of these herbivores are fish, sea urchins and molluscs (Morrison 1988). These herbivores are known to feed on a diverse range of algae, from erect algae to encrusting forms. Species that tend to feed on encrusting algae have specialized teeth that can scrape on hard substrates, and they have a diverse range of feeding behaviours (Mills et

al. 2000). They are capable of removing calcium carbonate particles from the reef substrate during feeding, and hence contribute to the reef structuring process as well (Bak 1990; Bak 1994; Carreiro-Silva and McClanahan 2001; Dumont et al. 2013; Glynn and Manzello 2015). Parrot fishes and sea urchins significantly contribute to the reef structuring process in many localities. Bellwood (1995) revealed that parrot fish species *Chlorus gibbus* and *C. sordidus* on the Great Barrier Reef erode 0.41 ± 0.07 kg m^2 a^{-1} and 0.0097 ± 0.0017 kg m^2 a^{-1} of $CaCO_3$ respectively. According to Glynn (1988), bio-erosion caused by the sea urchin *Diadema mexicanum* in Panama ranged from 5 to 20 kg m^{-2} a^{-1}. In French Polynesia *Diadema savignyi* and *Echinometra mathaei* are responsible for the erosion of 3.87 kg m^{-2} $a^{-1}$and 0.372 kg m^{-2} a^{-1} (Bak 1990). The composition of herbivore community is critical for structure and functioning of the reef (Burkepile and Hay 2008). Shifting from a fish herbivore community to sea urchin dominance is known to have negative impacts on reef health, with a possible increase in the level of bio-erosion (Brown-Saracino et al. 2007). Considering the immense impact on the system, it is essential to study and monitor these herbivore populations, such as sea urchins, prioritizing the geographical regions which are sensitive to rapid changes. Insights into their feeding ecology, population dynamics and recruitment patterns are vital, since many of these ecosystems are progressively subjected to anthropogenic impacts.

1.3.4.2 Predation

Predation is known to influence many habitats where a predatory species play a key role (Estes et al. 2010; Heithaus et al. 2008; McClanahan 2000; Stewart and Konar 2012). When predator pressure is high, it affects the appearance and functioning of ecosystems, and when it is low, it affects the patches of organisms or survival of individuals. A few studies have suggested that prey-predator interactions are site-specific, where certain prey is preferred by different predatory species in different locations (Young and Bellwood 2012). After catastrophic mortality reduced the *Diadema antillarum* population in Caribbean reefs in the early 1980s, recovery was observed only where the urchin's natural predator, the spotted spiny lobster, was overfished. Avoidance of spiny lobsters has been known to influence urchins' feeding behaviours (Kintzing and Butler IV 2014). Increased abundance and body size of lobsters and predatory fish are known to influence the recovery of macroalgae in New Zealand (Leleu et al. 2012). On the other hand, Johansson et al. (2013) documented that predation does not play a significant role in controlling *Echinometra mathaei* populations on Ningaloo Reef in Western Australia.

Relative effects on herbivory / predation (top-down control) or nutrients (bottom-up control) determine the community structure of a specific habitat (Ferrari et al. 2012). Seasonality and geographical location have the greatest influence on community structure of the ecosystem (Ferrari et al. 2012). In Caribbean reefs, although macroalgae and turf cover are controlled by herbivores, community structure is controlled by seasonal changes (Ferrari et al. 2012).

1.3.4.3 Availability of nutrients

The role of herbivores is crucial in any ecosystem and has a considerable control over the structure and function of many shallow habitats since energy transfer in a food web is initiated through herbivory. The availability of macroalgae is determined by nutrient levels and light availability. The impact of nutrient enrichment on macroalgae diversity, abundance and distribution have been subjected to study in many habitats globally (Boyer et al. 2004). A meta-analysis carried out by Worm et al. (2002) has shown very complex interactions between nutrient control and herbivores in the species distribution of primary producers. Previous studies based on manipulation of nutrient availability, herbivore pressure and different primary producer types across the world have shown anthropogenic alteration on nutrient availability can significantly impact on the proliferation of primary producers and species composition (Burkepile and Hay 2006; Burkepile and Hay 2009). However, the extent of impact depends on latitude, producer species and the productivity of the system. Rapidly increasing use of fertilizers has caused rapid changes in water quality in coastal waters (Bell et al. 2014; Brodie et al. 2012; Fabricius 2005). Excess nutrients added through terrestrial run off can accelerate macroalgae growth in regions where micro-nutrients are readily available (Moore et al. 2013; Moore et al. 2001; Roff and Mumby 2012).

In many tropical regions with the presence of adequate sunlight all year round, higher concentrations of nutrients can result in proliferation of macroalgae, changing food web dynamics. Thus, knowledge on rate of habitat response to these changes and process of recovery is essential. A habitat can compensate through increased intensity of herbivory, by means of increasing the feeding rate or increasing the abundance of the herbivores (Burkepile and Hay 2009).

Recent studies suggest that many coral-macroalgae phase shifts are human originated. Discharge of pollutants into the ocean has resulted in eutrophication in many systems, indirectly increasing bottom-up pressure on the system (Bell et al. 2014; Fabricius 2005). On the other hand, overfishing of competitive herbivore fish species and predatory fish species can change reef species composition and abundance,

eventually leading to single herbivore dominance in such habitats (Wallner-Hahn et al. 2015).

1.3.5 Predicting ecological interactions- food web analysis

It is hard to observe and study most of these interactions in the field of marine ecosystems, especially for subtidal coral reefs; one way to understand interactions among species is to analyse the feeding interactions. In many instances, food web analysis is used to predict the diet of species, determine the direction of energy flow and to assess the dependency of a species on others. Being the foundation for shallow benthic food webs, photosynthetic organisms, always transfer energy to upper tropic levels. Over past decades many traditional methods, such as gut content analysis of organisms, faecal matter analysis and direct observations from the habitats have been used to determine diet (Hyslop 1980). But the prediction of the real diet of species and their position in the food webs is difficult since identification of partially digested food particles in gut contents and faecal pellets is challenging (Baker et al. 2014). These traditional methods can predict the immediate diet of organisms. However, this information is not useful in predicting long term dietary habits and trophic level of the organism (Hussey et al. 2011).

Recent use of stable isotope analysis, identification of isotope signatures and distribution of isotope signatures in a chemical compound, has been very useful for ecological studies in analysing the trophic relationships in an ecosystem (Bearhop et al. 2004; Ben-David et al. 1997; Brett et al. 2009; Cabana and Rasmussen 1996; Davis et al. 2012; Fry 1991; Grey et al. 2001; Keough et al. 1996; Kharlamenko et al. 2001; Layman et al. 2012; Lepoint et al. 2004; Zanden and Rasmussen 2001). Stable isotope analysis can predict the diet of an organism over a long period of time and can be used for organisms with empty guts unlike in traditional methods (gut content analysis and faecal pellets analysis). In stable isotope analysis, ^{13}C and ^{15}N have been used to predict food sources and trophic levels of respective organisms (Davis et al. 2012; Jensen et al. 2012; Rodríguez-Barreras et al. 2015a; Vanderklift et al. 2006). Another tool used in recent trophic studies is fatty acid analysis. Animals produce limited numbers of fatty acids unique to their own taxa (Galloway et al. 2012); since most mono-saturated fatty acids are stored in fat stores with very little or no modification, fatty acid signatures are useful in identifying trophic relationships (Iverson et al. 2004; Ruess and Chamberlain 2010). A combination of both fatty acid signatures and stable isotope analysis is most effective in predicting prey and trophic relationships (Galloway et al. 2013; Guest et al. 2009; Kolts et al. 2013; Zieman et al. 1984). On the other hand, a combination of stable

isotope analysis with DNA analysis provides a better understanding of diet composition and food webs (Hardy et al. 2010).

The recent development of DNA sequencing technology has broadened the applications in many scientific fields and even has been used as a tool in ecological studies (Pompanon et al. 2012; Valentini et al. 2009). As a result, DNA markers have been successfully used for diet analysis and food web analysis (Blankenship and Yayanos 2005), and this technique has been used to predict diets of both carnivorous organisms (Barnett et al. 2010; Deagle et al. 2007) and herbivores (Bradley et al. 2007).

1.4 Ecological role of sea urchins on coral reefs

Sea urchins have been extensively studied during recent decades for their key role in benthic marine communities in different regions of the world (Adam et al. 2015; Bulleri et al. 1999; Byrnes et al. 2013b; Harrold and Pearse 1987; Harrold and Reed 1985; Humphries et al. 2014). Being grazers, the influence of sea urchins is immense as they control the growth of all organisms other than encrusting algae. High densities of sea urchins can remove competitive algae, reducing coral mortality due to algal overgrowth and allowing corals to increase in abundance (Bluhm et al. 2009). *Diadema antillarum* in Caribbean reefs (Adam et al. 2015; Bak et al. 1984), *Strongylocentrotus* sp. in the North Atlantic (Miller and Colodey 1983; Scheibling 1986; Scheibling et al. 1999; Scheibling and Lauzon-Guay 2010; Scheibling and Stephenson 1984), *Centrostephanus rodgersii* on the east coast of Australia (Ling 2008), *Heliocidaris erythrogramma* in Tasmania (Ling et al. 2010; Valentine and Johnson 2005) and *Evechinus chloroticus* in New Zealand (Andrew 1988) are well known for their dramatic impacts on habitats. They are capable of changing habitat structure and composition within a short period of time (Abraham 2007; Filbee-Dexter and Scheibling 2014; Flukes et al. 2012; Ling et al. 2009b; Scheibling et al. 1999).

The classic example of the impact of grazing by sea urchins comes from Caribbean reefs, where disease caused sudden mass mortality of *Diadema antillarum* in 1983-1984 (Carpenter 1990; Lessios et al. 1984b). *Diadema antillarum* was the keystone species in Caribbean reefs, being a major herbivore, corallivore and bio-eroder (Adam et al. 2015; Mercado-Molina et al. 2015). Thus, this species was critically important in the control and development of reef flora (Tuya et al. 2004). An estimated loss of 95-99% of the original sea urchin population during 1983-1984 directly affected reef ecology, resulting in increased biomass of macroalgae (Carpenter 1990). As a result, reduction in coralline algae covers and coral cover was evident. On the other

hand, the grazing pressure of herbivorous fishes was increased. Similarly, on the Atlantic coast of Nova Scotia, a disease outbreak eliminated the key herbivore *Strongylocentrotus droebachiensis* from reef systems (Scheibling 1986). Scheibling (1986) revealed an ecosystem shift from barren grounds to patches of dense kelp forest within three years of the urchin mass mortality. The absence of urchins was reported to allow production of kelp 313-900 g C m^{-2} a^{-1} from Vancouver island to the western edge of Alaska's Aleutian Islands (Wilmers et al. 2012). An urchin removal experiment from a sublittoral site in New South Wales, Australia resulted in rapid recovery of macroalgae in the region. Reintroduction of urchins to this habitat at a later stage has resulted in reduction of macroalgae (Andrew and Underwood 1993).

On the other hand, overgrazing by unusually dense aggregations of sea urchins in seagrass beds in Florida Bay changed the community structure, affecting infauna and epifauna as well as the physical properties of the sediment. A loss of fishery habitats, reduction of primary and secondary production and degradation of water quality and other long term effects in sea grass beds in outer Florida bay were documented by Rose et al. (1999). Adverse effects of seagrass grazing by *Heliocidaris erythrogramma* were also documented in Cockburn Sound, Western Australia (Langdon et al. 2011). As Andrew (1988) suggested, *Evechinus chloroticus* determines the characteristics of subtidal rocky reefs in northern New Zealand more than any other species, influencing the density and distribution of brown algae and molluscan herbivores negatively. Community structure of this urchin population is not influenced by the presence of predators such as rock lobster and predatory fish (Andrew 1988). *Heliocidaris erythrogramma* and *Centrostephanus rodgersii* on the south-eastern coast of Tasmania have had significant impacts on the local habitat, maintaining persistent barrens (Ling and Johnson 2009; Valentine and Johnson 2005).

In addition to being key herbivores, sea urchins also have been identified as bio-eroders (Appana and Vuki 2006; Bak 1990; Bak 1994; Dumont et al. 2013; Mokady et al. 1996), producers of particulate inorganic matter (Mamelona and Pelletier 2005) and habitat providers for other organisms (Gherardi 1991; Lowry and Pearse 1973; Patton et al. 1985). Sea urchins impact coral reef habitats through direct ingestion of reef carbonate material with the special feeding apparatus, "Aristotle's lantern" (Killian et al. 2011; Ma et al. 2008) and indirect erosion through spine abrasion. Removal of reef calcareous matter associated with urchin grazing is least understood and many past bio-erosion studies are restricted to tropical reefs. Sea urchin bio-erosion can alter reef structure and eventually impacting the other species hence: they are known as

"ecosystem engineers" (Bak 1994; Bronstein and Loya 2014; Mokady et al. 1996). The level of urchin bio-erosion can be equal to or exceed reef carbonate production of the reef (Alvarado et al. 2012; Bak 1994). The highest rate of echinoid bio-erosion, 7 kg $CaCO_3$ m^{-2} a^{-1}, was recorded in exposed habitats in Pongwe, Zanzibar (Bronstein and Loya 2014). Emerging evidence suggests that sea urchin bio-erosion impacts not only shallow reef communities but also deep cold water coral reefs (Stevenson and Rocha 2013).

Although sea urchin grazing is critical in determining kelp abundance in many geographic regions, it is not a universal phenomenon. Barrens are restricted to ecosystems where urchins have reached maximum densities with high average consumption potential. Further, variability in kelp abundance and variability of urchins consumptive potential accounts for barrens formation as well (Byrnes et al. 2013b). To evaluate the potential of urchins in influencing habitat and community structure, it is essential to study local factors such as their feeding ecology, population biology, and recruitment patterns, availability of predators and competitors, and local oceanographic conditions.

1.5 Sea urchin Biology

1.5.1 Sea urchin feeding

Sea urchins are capable of selective feeding, altering macroalgae species composition and promoting species diversity in moderate densities. Since carbonate erosion is unavoidable with their feeding, over 70% of their diet is composed of calcium carbonate (Carreiro-Silva and McClanahan 2001; Mills et al. 2000). The organic component of their diet is mostly determined by the local diversity and abundance of algae and seagrass (Murillo-Navarro and Jiménez-Guirado 2012). Most temperate urchins associated with kelp forests totally depend on kelp while urchins in barrens depend mostly on crustose coralline algae (Harrold and Pearse 1987; Harrold and Reed 1985; Wilmers et al. 2012). In general, urchins are known to be herbivorous in nature, yet few species have exhibited variations in dietary habits. *Phyllacanthus irregularis* and *Centrostephanus tenuispinus* in Western Australia are capable of feeding on other organisms, showing a more omnivorous diet (Vanderklift et al. 2006). Although *Strongylocentrotus droebachiensis* is known as a herbivore in many habitats, this species is known to feed on ascidians, changing the overall distribution pattern of the ascidian species in the Gulf of Maine (Simoncini and Miller 2007). *Diadema setosum* in Hong Kong has been observed actively feeding on coral polyps, causing community

level destruction (Qiu et al. 2014). Irrespective of their feeding preferences, being intense grazers, urchins alter the habitat structure and species diversity of the habitat.

Sea urchin species that tend to co-occur in the same habitat are known to use different resources (Bulleri et al. 1999; Hill and Lawrence 2003). *Paracentrotus lividus* and *Arbacia lixula* co-occur in the Mediterranean region and exhibit different dietary habits; only *Paracentrotus lividus* is capable of feeding on coarsely branched erect algae and drifting algae. Urchins have a remarkable ability to respond to different conditions in the environment since they are voracious feeders. Since, they are directly influenced by food availability; changes in urchin populations are mostly associated with changes in macroalgae cover.

1.5.2 Sea urchin population size regulation

Change in sea urchin population size is critical for the long term stability of the community structure of the local habitat (Adam et al. 2015; Brady and Scheibling 2005; Carpenter 1990; Feehan and Scheibling 2014; Hughes et al. 1987; Lessios 1995; Ling et al. 2015; Qiu et al. 2014; Scheibling and Lauzon-Guay 2010). Knowledge on population fluctuations and size structure is essential to assess the intensity of grazing and bio-erosion in an ecosystem. Numerous studies focused on sea urchin population dynamics have revealed many factors that can cause changes in urchin population density (Carpenter 1988; Wilmers et al. 2012).

The optimum urchin density that can be sustained by each habitat is unique to each system in response to habitat characteristics. Barnes and Hughes (1998) observed that a density of 37 urchins m^{-2} appeared to be at equilibrium with the kelp in Nova Scotia in Canada. It is essential to understand natural equilibrium and threshold levels of an ecosystem for restoration and conservation with the current rate of the changing environment.

Conditions such as eutrophication, which causes rapid growth in macroalgae, can directly influence urchin populations. Sea urchins can adjust their body size, population size and growth rate in response to food availability. Sea urchin density and body size are the two most important determinants of the spatial pattern of the population (Hagen and Mann 1994). Levitan (1989) recorded a significant inverse relationship between mean urchin body size and population density and suggested that urchins have the ability to adjust their skeletal body size towards this relationship. When food is scarce, some echinoids also have the ability to increase the size of their Aristotle's lantern compared to the body size (Ebert 1980; Levitan 1991). Thus, sea urchins in barrens feed mainly on microbial films and encrusting coralline algae and

tend to be smaller than those in kelp beds or feeding fronts (Coppard and Campbell 2005a; Johnson and Mann 1982; Ling and Johnson 2009; Ling et al. 2015; Scheibling et al. 1999). The inverse relationship between body size and population density in response to food availability have been documented in many geographical locations (Johnson and Mann 1982; Ling and Johnson 2009; Tuya et al. 2004). Size dependent distribution within the habitat depending on the water depth in response to hydrodynamic conditions has also been recorded by Freeman (2003).

Population variation of sea urchins in a given space is also closely related to predation (McClanahan 1998). The high levels of predation result in the variations in population size structure and low spatial variation. Many predatory fish species, lobsters and otters are known to impact on urchin populations in different geographical regions, although mostly predators appeared to be site specific (Abdo et al. 2012; Bonaviri et al. 2009; Kintzing and Butler IV 2014; Stewart and Konar 2012; Wallner-Hahn et al. 2015; Young and Bellwood 2012). As both lobsters and fish are harvested commercially worldwide, practices of fishing these groups directly influence the sea urchin population density and structure (Lafferty 2004; McClanahan et al. 1994). Current trends in overfishing in some regions can have dramatic impacts due to the expansion of sea urchin populations with the absence of the top predators.

Overfishing has been shown to have dual impacts on sea urchin populations, reducing competitive herbivore fishes and reducing predatory fishes. Changing from multispecies herbivory to single species herbivory, lack of predation or additive effect of both have led to thriving urchin populations and changes in size structure of the population (Behrens and Lafferty 2004; Ling et al. 2009a; Rodríguez-Barreras et al. 2015b; Wallner-Hahn et al. 2015). Behrens and Lafferty (2004) have recorded bimodal size frequency distributions of *Mesocentrotus franciscanus* and *Strongylocentrotus purpuratus* within Channel Island National Park and unimodal size frequency distributions in fished areas. Further, sites within Channel Island National Park were more likely to be kelp forests, and fished sites were more likely to be barrens.

Mesocentrotus franciscanus (red urchin) and *Strongylocentrotus purpuratus* (purple urchin) are important members of southern Californian kelp bed communities (Tegner and Dayton 1981). Differences in their test diameters and relative spine lengths have resulted in variations in exposure to their predators, behaviours and habitat partitioning. In the case of *Mesocentrotus franciscanus* populations, it is the intermediate size category of urchins that is more vulnerable, as small individuals seek protection under the spine canopy of larger individuals. Tegner and Dayton (1981)

revealed that this population is strongly bimodal in the presence of predators such as lobsters and California sheep head. However, small *Mesocentrotus franciscanus* are found in a variety of micro-habitats, and they do not utilize the spine canopy association. Hence they show unimodal population distribution.

Recruitment patterns also play a key role in population regulation in many sea urchin populations. Studying reproductive periodicity is essential to predict on population dynamics of a particular species.

1.5.3 Sea urchin reproduction

The knowledge of reproductive seasonality is essential to understand population changes. A review of Giese and Pearse (1974) on timing and patterns of reproduction in marine invertebrates mentions that species have broad latitudinal ranges have varying breeding seasons. Generally, when the environment is highly seasonal reproduction is limited, and in the tropics, it is extended. Reproductive periodicity varies greatly, depending on the species and on environmental conditions (Bronstein et al. 2016). In addition, variations due to spatial and temporal aspects should be considered, thus locality and seasonality of recruitment need to be studied carefully.

Some sea urchin species exhibit only one annual reproductive cycle while others are known to have several reproductive cycles within one year (Byrne et al. 1998; Gonor 1973; González-Irusta et al. 2010; Lawrence 2013; Molinet et al. 2012). *Tripneustes gratilla* in the Indo-West Pacific exhibits an annual reproductive cycle with variable intensity (Vaitilingon et al. 2005). *Echinometra mathaei* and *Diadema savignyi* on the South African eastern coast have annual reproductive cycles spawning restricted summer. *Diadema savignyi* has a distinct annual cycle with synchronization, while *Echinometra mathaei* shows very weak synchronization during the spawning period (Drummond 1995). On the other hand, reproductive periodicity was less defined on the less seasonal Panama, Caribbean coast (Lessios 1981).

Spawning is induced by different factors. Past studies revealed that spawning is induced by photoperiod and light intensity (Brogger et al. 2010; Byrne et al. 1998; Shpigel et al. 2004), time of the day (Pearse et al. 1998) temperature (Bronstein and Loya 2015; Shpigel et al. 2004), phytoplankton/chlorophyll *a* (Gaudette et al. (2006), lunar cycle (Coppard and Campbell 2005b), tides/currents (Boolootian et al. 1959) and salinity (Giese et al. 1964). For the majority of temperate urchins, spawning is mainly influenced by photoperiod and light intensity; thus spawning is seasonally restricted to winter or summer. Although few authors suggest that sea water temperature does not play a central role in regulating reproductive cycles in Indo-Pacific sea urchins

(Drummond 1995; Pearse and Cameron 1991), laboratory experiments show a temperature increment can induce the spawning of many urchins. Evidence suggests that temperature plays a major role in larval development and settlement (Hart and Scheibling 1988; Hernández et al. 2010; Pecorino et al. 2013b).

Past studies have revealed that availability of food sources directly influence gonad development (Byrne et al. 1998). Differences in gonad weight of individuals from kelp beds and grazing fronts compared to individuals from barrens have been clearly observed (Byrne et al. 1998; Meidel and Scheibling 1998). Differences in diets and food availability can contribute to differences in gonad indices between populations of the same species in different habitats (Byrne 1990; Byrne et al. 1998; Pecorino et al. 2013a). Lower reproductive output was observed in barrens which have limited food (Byrne et al. 1998). Meidel and Scheibling (1998) suggested that diet influences reproductive maturation and growth rate of *Strongylocentrotus droebachiensis*. This has been demonstrated with many other species in different localities as well (Cook and Kelly 2007a; Jacquin et al. 2006; McBride et al. 2004; Shpigel et al. 2005).

1.6 Temperate sea urchins

Temperate sea urchins gained the attention of many scientists due to their key role in kelp forests and their value in fisheries. *Strongylocentrotus droebachiensis, S. purpuratus* and *Lytechinus variegates* (Moore et al. 1963) have been subjected to many field-based observations and laboratory experiments due to their key roles in kelp forests and sub-tidal reefs in temperate regions of the northern hemisphere (Cowen 1983; Himmelman and Steele 1971; Keats et al. 1984; Norderhaug et al. 2014; Pearce and Scheibling 1991; Quinn et al. 1993; Tegner and Dayton 1981; Thompson 1983). *Centrostephanus rodgersii*, *Heliocidaris erythrogramma* and *Evechinus chloroticus* have been greatly acknowledged for their critical role in southern temperate habitats (Australia and New Zealand) and for their value as a fishery product (Andrew and MacDiarmid 1991; Andrew 1988; Byrnes et al. 2013a; Hill et al. 2003; Johnson et al. 2011; Kailola 1993). *Centrostephanus rodgersii* is well studied for its recent range expansion towards poles and it is intensively studied in newly established habitats. (Johnson et al. 2005; Ling and Johnson 2009; Ling et al. 2008; Ling et al. 2009b; Pecorino et al. 2012; Pecorino et al. 2013a; Pecorino et al. 2013b).

1.7 *Centrostephanus* spp.

Centrostephanus rodgersii and *Centrostephanus tenuispinus* in Australia are closely related and distributed in eastern, southern and western Australian waters.

Centrostephanus rodgersii is a highly successful grazer and is distributed in a wide range of habitats, initially restricted to the east coast of Australia (Andrew and Byrne 2007; Byrne et al. 1998). This species has gained substantial attention due to its range extension from south-eastern Australian to Tasmanian coastlines (Johnson et al. 2011; Ling et al. 2009b) and in New Zealand waters towards the southern seas during the last four decades (~160 km decade^{-1}). It is known that there has been an increase in sea surface temperature of 1.5°C in southern higher latitudes since the 1960s, which is a substantial change for temperate marine species. Thus, this shift is in response to the increase in the southward and eastward flow of the East Australian Current, which has resulted in increased sea surface temperature (Ling et al. 2009b; Ridgway 2007). Due to the long lived pelagic larval stage, these urchins are capable of migrating southwards within thermal threshold levels (12C°), despite the discontinuous nature of reefs (Huggett et al. 2005; Ling et al. 2009b). Further, Pecorino et al. (2013b) suggested the potential of future expansion of distribution range of this species with future ocean warming conditions.

In many temperate reef ecosystems, sea urchins exert immense pressure on benthic habitats. They are capable of preventing the growth of all macroalgae except crustose coralline algae. *Centrostephanus rodgersii* is well known to create barrens on the east coast of Australia and Tasmania (Andrew and Underwood 1993; Andrew and Byrne 2007). These barrens persisted in 2-10 m depth range in New South Wales, around 10-20 m in northern Tasmania and around 15-35 m in east Tasmania (Johnson et al. 2005). Stability of these barrens largely depends on density and recruitment of urchins (Andrew and Underwood 1989a). Barrens are more persistent in boulder substrates in Tasmania. Thus, nearly 50% of rocky habitats on the east coast of Tasmania are likely to be influenced by urchins (Johnson et al. 2005). Many urchin barrens are stable and only reversed by mass mortalities of urchins (Andrew 1991). Total removal of *C. rodgersii* from the habitats is known to reduce percentage cover of crustose corallines and loss of the barrens within a few months in New South Wales (Andrew and Underwood 1993). Partial removal of urchins (removal of 33% and 66% of the original population) caused reduction of the size of barren patches. Only 33% of natural density is needed to maintain barren areas (Andrew and Underwood 1993; Hill et al. 2003).

Newly established *Centrostephanus rodgersii* barrens are known to influence the abundance of abalone (*Haliotis rubra*) and rock lobster (*Jasus edwardsii*). Clear negative relationships have been observed in the density of abalones and rock lobsters

with respect to the density of urchins (Johnson et al. 2005). Since primary production is low in barrens, competition between abalone and urchins is inevitable. Removal of *C. rodgersii* from barrens in New South Wales has increased abalone density considerably. Due to its immense influence on eastern and southern Australian and Tasmanian reefs, *C. rodgersii* has been extensively studied for its feeding behaviour (Andrew and O'Neill 2000; Andrew and Underwood 1993; Andrew and Byrne 2007; Ling and Johnson 2009), reproductive biology (Byrne et al. 1998; King et al. 1994; Ling et al. 2008; Pecorino et al. 2013a) and response to global climate change (Foo et al. 2012; Johnson et al. 2011; Ling et al. 2009b; Pecorino et al. 2013b). On the other hand, its congener *C. tenuispinus* on the west coast of Australia is understudied.

1.7.1 *Centrostephanus tenuispinus* in Western Australia

Centrostephanus tenuispinus has been recorded from the Houtman Abrolhos Islands (29°S) to Fremantle (32°), along the west coast of Australia and in southern Australian waters (Great Australian Bight) (Fell 1975). This species is known to be present in many habitats in the Perth region: Rottnest Island, Fish Rocks, Mewstone Rocks, Stragglers Rocks, Minden Reef (Richards et al. 2016) and Hall Bank reef (Thomson and Frisch 2010). Fell (1975) recorded *Centrostephanus tenuispinus* on the southern coast of Australia. Yet no information is available on recent distribution patterns of *C. tenuispinus* elsewhere in southern Australia.

Centrostephanus tenuispinus has black or reddish brown or pale greenish coloured spines. It is distinguished from *C. rodgersii* by the presence of prominent tubercles on apical plates. Recent studies on *C. tenuispinus* diet suggest that they consume a considerable proportion of animal tissues (sponges and ascidians) compared to other co-occurring sea urchin species. Stable isotope analysis by Vanderklift et al. (2006) on the lantern muscles of *C. tenuispinus* indicated high $\delta^{15}N$ values, which is unusual for herbivores. The high percentage of sand and rock fragments (9.5 ± 2.7 %) in urchin guts, compared to other co-occurring species is indicative of active grazing.

Knowledge on *C. tenuispinus* is restricted to only a few studies conducted on food web ecology (Vanderklift et al. 2006), predator interactions (Cook and Vanderklift 2011) and population density (Thomson and Frisch 2010). These studies suggest similarities in grazing habits exhibited by the congener *C. rodgersii* on the eastern Australian coast. Therefore, filling the knowledge gap on the ecological role of *C. tenuispinus* on reef habitats is essential for future conservation efforts.

1.7.2 *Centrostephanus tenuispinus* population in Hall Bank reef

The Western Australian coast is unique among western coastlines with the influence of the Leeuwin current, which carries warm tropical water poleward. The Leeuwin Current extends from Northwest Cape (22°) towards Cape Leeuwin (34°) (Cresswell and Golding 1980). It is driven by the Indonesian Through-flow, which carries warm water from the Pacific Ocean to the Indian Ocean (Feng et al. 2009a; Feng et al. 2009b). The Leeuwin Current is a convection current, and the strength of the current varies depending on prevailing wind (Godfrey and Ridgway 1985). It is more dispersed at the Abrolhos Islands and Perth, but stronger at Cape Leeuwin (34°) (Godfrey and Ridgway 1985). Since the Leeuwin current is responsible for the poleward movement of warm water from the tropics, it is responsible for the presence of tropical species at higher latitudes, and it is unique when compared to any other subtropical currents.

The Leeuwin Current is known to exhibit seasonal and inter-annual variability. It is strong in the austral winter and weak in the austral summer. Further, the Leeuwin Current is stronger during La Niño conditions and weaker during El Niño (Feng et al. 2009a; Feng et al. 2009b). Since this current suppresses the upwelling of cool nutrient rich water, nutrient deficient warm water is inevitable in shallow waters of Western Australia reducing the productivity on the continental shelf.

Hall Bank reef (32°2.002′S and 115°42.957′E) is unique for its high hard coral cover (mean = 52.6 ± 4.65 %) where the majority of subtidal reefs in Perth area are dominated by macroalgae (Thomson and Frisch 2010). Hall Bank reef is located closer to the 20°C isotherm and high percentage of hard coral cover, is quite unusual for this latitude. In contrast to the typical reefs in the region, soft corals and macroalgae in this habitat are scarce. The high density (5.0 ± 0.81 m^{-2}) of the sea urchin *Centrostephanus tenuispinus* is assumed to be the main reason for the absence of macroalgae in Hall Bank reef (Thomson and Frisch 2010). Among the 14 documented coral species on Hall Bank reef, 11 species have widespread distributions in both tropical and temperate latitudes. One subtropical species (*Goniastrea australensis*) is uncommon at higher latitudes, and two species (*Coscinaraea mcneilli* and *Coscinaraea wilsoni*) are temperate corals. The presence of coral species from different bio-geographical regions makes Hall Bank reef unique among other higher latitude reefs.

1.8 Thesis Aims:

The ecological role of *Centrostephanus tenuispinus* in reef habitats has not been explored in previous studies. Lack of knowledge on the biology and ecology of *C.*

tenuispinus hampers proper understanding of the functioning of *C. tenuispinus* inhabiting reefs. Limited knowledge from the few studies on this particular species suggests their resemblance to the congener *C. rodgersii* on the east coast of Australia. Considering the great influence of *C. rodgersii* on east coast of Australia and newly established habitats in Tasmania and New Zealand (Johnson et al. 2005; Ling 2008; Ling et al. 2009b), baseline knowledge on the biology and ecology of *C. tenuispinus* will benefit management of west Australian reefs with this species.

This study was mainly focused on two directions; filling in the gaps on baseline data on *C. tenuispinus* and assessing the role of *C. tenuispinus* population in coral dominated Hall Bank reef. Further, population size structure and feeding habits were compared with a *C. tenuispinus* population in nearby macroalgae-dominated Minden Reef. Some of the curious recurring questions on *C. tenuispinus* are: how is this species sustained in reefs such as Hall Bank reef in the absence of macroalgae? Are they capable of creating barrens? This study attempts to fill the knowledge gaps of the feeding ecology of *C. tenuispinus* by identifying and quantifying the gut contents. Quantifying bio-erosion integrated with feeding will be helpful in understating their level of impact on the reef structuring process.

Personal observation by Thomson and Frisch (2010) indicates that herbivorous fish are scarce in this particular habitat. Having similar conditions such as light and nutrients, how coral outcompetes macroalgae in this location is poorly understood. In this scenario, the role of major predators, such as predatory fish must also be considered. Although rock lobsters are known to prey upon urchins in other geographical locations, Dumas et al. (2013) indicated that the western rock lobster mainly preys upon decapods, and urchins are less preferred. Therefore, it is important to look into the food web dynamics with special reference to *Centrostephanus tenuispinus* as the dominant grazer in this system. Information about the temporal variation of the urchin population and reproduction will help us to understand how they function in the system. Studying the impacts of herbivory on the reef community is essential for a proper understanding of the system (Hernández et al. 2008). Therefore, this study will give some insights into indirect effects of the removal of sea urchins from marine ecosystems.

The presence of sea urchins in Hall Bank reef is assumed to be the main reason for the observed high coral cover. Does *Centrostephanus tenuispinus* facilitate coral growth by grazing? Other possible reasons for the low macroalgae cover could be the low nutrient levels in this habitat. Despite the presence of *C. tenuispinus* at nearby

Minden Reef, it is dominated by macroalgae and exhibits typical features of temperate reefs. Importantly, both reefs are located just one kilometre away from the mouth of the Swan River, which carries land run off from over 120,000 km^2 of catchment; it is essential to look at the impacts of river outfall on the reef diversity as well. When river outfall is closer to a coral reef, it subsequently increases the turbidity of adjacent waters influencing on the reef functions specifically by destructing hard corals (Burke et al. 2011). Burke et al (2011) identified the dominant threats to the shallow reefs as marine-based pollution and watershed-based pollution. Destruction of coral reefs has been recorded in Hervey Bay, Queensland due to river run-off. Mortality of corals was highest close to the mainland with increased level of turbidity and nutrient levels (Butler et al. 2013).

Knowledge on feeding ecology, population biology and reproductive biology of *Centrostephanus tenuispinus* will give insights into its role at Hall Bank reef. The main objective of this study is to evaluate the role/contribution of *Centrostephanus tenuispinus* in structuring and functioning of Hall Bank reef. To achieve this main objective, this study was carried out in four aspects:

1. Present population status and size distribution of *Centrostephanus tenuispinus* at Hall Bank reef and Minden Reef with respect to the environmental/substrate characteristics (Chapter 2)
2. Reproductive periodicity of *C. tenuispinus* (Chapter 3)
3. Studying the feeding/food web ecology of C. *tenuispinus* (Chapter 4)
4. Role of *C. tenuispinus* as a bio-eroder (Chapter 5).

Chapter 2 – Population status and size distribution of *Centrostephanus tenuispinus*: Hall Bank reef vs Minden Reef

2.1 Introduction

2.1.1 Sea urchins as keystone species

Sea urchins are keystone species in many shallow marine benthic ecosystems (Adam et al. 2015; Carpenter 1988; Harrold and Pearse 1987; Lawrence 1975; Lawrence 2013; Steneck 2013). The extensive grazing nature of regular echinoids enables them to play a crucial role in the habitat they inhabit. Although urchins are well known for promoting coral recruitment, they are capable of overgrazing macroalgae and converting habitat to crustose coralline-dominated barrens at high population densities (Bak et al. 1984; Byrnes et al. 2013b; Filbee-Dexter and Scheibling 2014; Johnson et al. 2004; Ling and Johnson 2009; Ling et al. 2009a; Mayfield and Branch 2000; Pederson and Johnson 2006). The dominance of macroalgae over corals in the absence of urchins has been documented in many regions of the world (Adam et al. 2015; Carpenter 1990; Girard et al. 2012; Lessios 1988a; Scheibling 1986). *Diadema antillarum* on Caribbean reefs and *Strongylocentrotus droebachiensis* on the Atlantic coast of Nova Scotia are classic examples of the massive ecological shifts caused by mass mortality of sea urchin populations due to disease conditions (Carpenter 1990; Scheibling 1986). Increased biomass of macroalgae dominating over coral and changing species composition has been documented in these respective habitats. In addition to population size, the level of impact of urchin grazing depends on factors such as feeding mechanisms and urchin size as well. Further, geographical and spatial variation in grazing intensity has been recorded in past studies (Lawrence 2013). Considering their ability to alter benthic community composition and structure, estimating population size is essential when it comes to management of sea urchin inhabiting reefs.

2.1.2 Phase shifts from kelp forest to barrens- urchins as mediators

Reef communities in high latitudes are in the range of marginal conditions in many aspects for coral growth (Beger et al. 2014; Veron 1995). The distribution of reef structure and communities vary depending on local factors such as light availability, temperature and nutrient levels. Phase shifts between coral domination and macroalgae/kelp domination are mostly mediated by herbivores. Sea urchins, as dominant herbivores, exert top-down control over kelp forests in many temperate reefs (Byrnes et al. 2013b; Filbee-Dexter and Scheibling 2014; Harrold and Pearse 1987; Pearse and Hines 1987; Scheibling 1986; Scheibling and Stephenson 1984).

Formation of crustose coralline-dominated barrens due to urchin overgrazing is well documented in many geographical locations (Bonaviri et al. 2011; Connell and Irving 2008; Flukes et al. 2012; Ling et al. 2009a; Ling et al. 2015), yet the mechanisms of formation and extension of these barrens are poorly understood, possibly due to the variability in factors operating in different geographical locations (Byrnes et al. 2013b). Species-specific feeding habits/ecology are known to impact on the stability and extension of these barrens. A direct linear relationship between *Strongylocentrotus droebachiensis* density and extension of barrens was documented in Nova Scotia, Canada (Feehan et al. 2012). Since strongylocentroid urchins respond to food cues from macroalgae and are known to aggregate using chemosensory stimuli, they are capable of expanding barrens with high feeding rates (Mann et al. 1984). In contrast, *Centrostephanus rodgersii* on the east coast of Australia does not respond to chemical stimuli from macroalgae (Flukes et al. 2012). Since, they exhibit homing behaviour they form stable barren patches and density in these patches are known to be stable. The only way of expanding barrens is by means of increasing the urchin population density (Flukes et al. 2012). Some urchin species play a dominant role in the formation and extension of barrens over other co-existing urchin species (Bonaviri et al. 2011; Guidetti 2006).

Ecosystems in which kelp abundance is driven by urchin consumption are known to have achieved high maximum urchin densities, high average urchin consumptive potential, high variability of kelp abundance, and high variability in urchin consumptive potential (Byrnes et al. 2013b). Kelp interaction, factors controlling urchins' consumptive potential and inherent variability within systems leading to higher biomass of urchins also need to be considered in predicting the susceptibility of a certain region for barren formation (Byrnes et al. 2013b). In areas where conspicuous barrens are absent, yet urchins are present, local factors could be not only preventing destructive grazing, but also controlling urchin density. It is therefore essential to focus on species-specific studies of population dynamics and feeding ecology of sea urchins at local scales.

2.1.3 Factors regulating sea urchin population density

In general, the population size of grazers is controlled by the availability of food/algae and predatory pressure (Carpenter 1984; Heithaus et al. 2008; McClanahan 1999a). Yet, the sudden disappearance of grazer populations due to disease conditions has also been documented (Carpenter 1990). As herbivores, sea urchins readily respond to increases in their food sources: algae and seagrass. Thus, any factor that increases

algae cover can indirectly favour the urchin population; specifically, conditions with high nutrients are well known to impact algal abundance (Burkepile and Hay 2006). Since food preferences and feeding mechanisms can vary depending on the species, the extent of their grazing varies as well (McClanahan 1999a; Stimson et al. 2007; Vanderklift et al. 2006).

Predation also has an effect on urchin habitats at different levels. When operating at the largest scale, predation affects the appearance and functioning of ecosystems, and at the smallest scale, it affects patches of organisms or survival of individuals. Predation plays a major role in many habitats where predatory species play keystone roles (McClanahan 2000). In general, lobsters, sea otters and few fish species prey upon sea urchins; however, dominant predator species vary depending on locality (Table 2.1).

Table 2.1 Summary of main sea urchins predators at different geographical regions

Sea urchin Species	Location	Predator	Reference
Echinometra viridis	Caribbean reefs	*Calamus bajonado* (Jolthead porgy)	McClanahan 1998)
		Canthidermis sufflamen (Queen trigger fish),	
		Balistes vetula(Ocean triggerfish),	
		Lachnolaimus maximus (Hogfish)	
		Cassis madagascariensis	
Strongylocentrotus purpuratus	Channel Island	Lobsters	Lafferty 2004
Mesocentrotus franciscanus and *Strongylocentrotus purpuratus*	San Diego, Southern California	Spiny lobster (*Panulirus interruptus*) and Californian sheep head fish (*Semicossyphus pulcher*)	Tegner and Dayton 1981
Mesocentrotus franciscanus	Pacific Groove, California	*Pycnopodia helianthoides* (sea star)	Pearse and Hines 1987
Strongylocentrotus spp.	Alaska	*Enhydra lutris* (Sea otter)	Duggins 1980; Estes and Palmisano 1974
Paracentrotus lividus	Mediterranean	Sparids (*Diplodus sargus* and *D. vulgaris*)	Sala 1997
		labrids (*Coris julis* sp.)	
Eucidaris galapagensis	Galapagos Islands	hogfish, *Bodianus diplotaenia*, trigger fishes, *Pentaceraster cumingi*	Dee et al. 2012)
Echinometra viridis	Belize	Wrasses and hog fish	Brown-Saracino et al. 2007

Due to the immense impact of predation on controlling urchin population, overfished habitats tend to have high densities of urchins and signs of degradation (Hernández et al. 2008; Ling et al. 2009a; Tuya et al. 2004).

2.1.4 Factors controlling sea urchin population size distribution

Sea urchin are capable of adjusting their body sizes in response to food availability, population density, predatory pressure and habitat characteristics (Hernández and Russell 2010; Levitan 1991). The sea urchin *Anthocidaris crassipus* exhibits a size-dependent distribution; on steep rocky slopes the test diameter of urchins increased as water depth increased. This may indicate resource partitioning although the influence of hydrodynamic conditions on test size may also mediate this segregation (Freeman 2003).

2.1.4.1 Population density

Sea urchins have a remarkable ability to adjust body size in response to their density (Levitan 1991; Levitan 1989; Tuya et al. 2004). Inverse relationships between mean body size and population density have been observed in populations of *Diadema antillarum* where at high densities, small / intermediate sized urchins are more abundant and at low densities, large sized individuals are dominated (Levitan 1989; Tuya et al. 2004). Further, physical complexity of the substrate is known to be the main reason for patchiness of *D. Antillarum* in the Canarian Archipelago (Tuya et al. 2004).

Sea urchins in barrens are known to be in high density and also small to intermediate size. In contrast urchins inhabiting kelp forests are larger in size and lower density (Hill et al. 2003; Wing 2009). Juveniles of *Strongylocentrotus droebachiensis* in barrens in Newfoundland grow slowly and growth is known to stop to a stationary mode when food is scarce and urchins are in high density. Larger urchins with high mobility exhibit higher growth rates with more access to food (Himmelman 1986).

2.1.4.2 Food availability

As grazers, sea urchins are always ready to feed and are directly influenced by food availability and habitat characteristics. They are capable of adjusting their body sizes, population sizes, and growth rate in response to food availability (Levitan 1991). When food is limited, some echinoids are capable of enlarging their Aristotle's lantern compared to their body size (Coppard and Campbell 2005a; Ebert 1980; Ebert et al. 2014; Levitan 1991). This altered state of the body can be used to detect food limitations in the environment (Levitan 1991). Urchins are capable of adjusting skeletal test, body mass and gonads, proportionally reducing the energy costs. When these

echinoids inhabit food-constrained barrens, they mainly depend on microbial films and encrusting coralline algae (Johnson and Mann 1982); thus, they tend to be smaller than those in kelp beds or feeding fronts (Ling and Johnson 2009; Scheibling et al. 1999). On the other hand, urchins inhabiting barrens are known to have larger lanterns in response to food scarcity (Agnetta et al. 2013). *Centrostephanus rodgersii* in Tasmania has shown clear differences in size and morphology in response to food availability. Urchins of a relatively large size with a thick test, rapid growth and short spines inhabit macroalgae beds, while urchins with smaller body size, slower growth and thinner test are known to persist on the barrens habitat (Ling and Johnson 2009).

2.1.4.3 Predation

Predation is known to control the population size of many herbivore populations in many marine benthic ecosystems (Rodríguez-Barreras et al. 2015b; Sala and Zabala 1996; Tegner and Dayton 1981). Fish predators are known to affect population size structure of sea urchins (Rodríguez-Barreras et al. 2015b; Sala and Zabala 1996). Relative abundance of medium sized *Diadema antillarum* has been known to increase in the absence of their main predators *Thalassoma bifasciatum* and *Halichoeres bivittatus* in Puerto Rico (Rodríguez-Barreras et al. 2015b). A bimodal size frequency structure has been observed in urchin populations of *Mesocentrotus franciscanus* in San Diego, California in response to predatory pressure. Since *Mesocentrotus franciscanus* has long spines, small urchins are protected under spine cover of larger urchins, leaving intermediate urchins to be vulnerable to predation. In contrast, the population structure of *S. purpuratus* is unimodal, urchins having shorter spines (Tegner and Dayton 1981); thus, large urchins are incapable of providing refuge for small urchins. A bimodal population size structure has been recorded for *Paracentrotus lividus* in the Mediterranean due to predation (Sala and Zabala 1996).

2.1.5 *Centrostephanus tenuispinus* population at Hall Bank reef, Western Australia

Each reef has its unique mechanisms for operating and controlling ecological shifts between coral domination and macroalgae domination whether it is low latitude, tropical or high latitude, temperate. The same environmental factor may affect an organism differently with the dimension of time (Hughes and Connell, 1999), making it difficult to generalize situations. Therefore, studying the mechanisms controlling the reefs on an individual basis is essential to understand the ecology of each reef.

Hall Bank reef in Western Australia is unique among other reefs for many reasons. This reef is located near the 20°C isotherm, providing marginal conditions for

growth of many scleractinian tropical corals. Hall Bank has the highest coral cover (mean 52.6 ± 0.45 %) recorded beyond 29°latitude and is dominated by massive corals. The absence of macroalgae and soft corals is unusual for a reef in this latitude; many of the adjacent reefs are macroalgae-dominated. The high density of *Centrostephanus tenuispinus* on this reef is thought to be the main reason for the absence of macroalgae in the system (Thomson and Frisch 2010). In contrast, Minden Reef is dominated by macroalgae and soft coral and is considered to be a highly productive system. *Centrostephanus tenuispinus* is known to inhabit both reefs in different scales. Differences in population densities and size structure of its co-species *C. rodgersii* with respect to food availability have been observed and well-studied on the eastern coast of Australia and Tasmania. *C. rodgersii* is known to form barrens; is *Centrostephanus tenuispinus* capable of forming barrens? Vanderklift et al. (2006) suggested that *C. tenuispinus* tends to be an active grazer and an omnivore, which shows affinities with its co-species on the east coast. Different locally operating factors can be attributed to the urchin's tendency for overgrazing (Byrnes et al. 2013). Thus, species-specific studies based on individual reefs are essential to understanding the long term population dynamics of a species. Further, it is essential to know population status under normal/natural circumstances to predict the impact of anthropogenic impacts or changes due to climate change (Lessios 2013). The scarcity of data on long-term population dynamics of many sea urchin populations hampers predictions under anthropogenic influences (Uthicke et al. 2009). There are only a few studies of the *Centrostephanus tenuispinus* population in Western Australia, focused on feeding ecology and two studies on population density. Information on temporal variation of population and size structure is not available for this particular species. To assess the ecological role of this particular species it is essential to fill the knowledge gap on the population biology of the species. Also, knowledge on population size structure is essential for better understanding of role of urchin size in structuring the reef communities. Thus this chapter is focused on following objectives:

1.1 Estimating the temporal variation of density of *Centrostephanus tenuispinus* populations at Hall Bank reef (low productive) and Minden Reef (high productive) with respect to substrate composition.

1.2 Determination of the population size distribution of *C. tenuispinus* at Hall Bank reef and Minden Reef.

1.3 Assess the impact of grazing of *C. tenuispinus* on species composition of Hall Bank reef.

2.2 Methodology

2.2.1 Study site

The study was carried out at two study sites: Hall Bank reef (32°2.002´S and 115°42.957´) and Minden Reef (32º 04.320′S and 115º 43.782′E) (Figure 2.1).

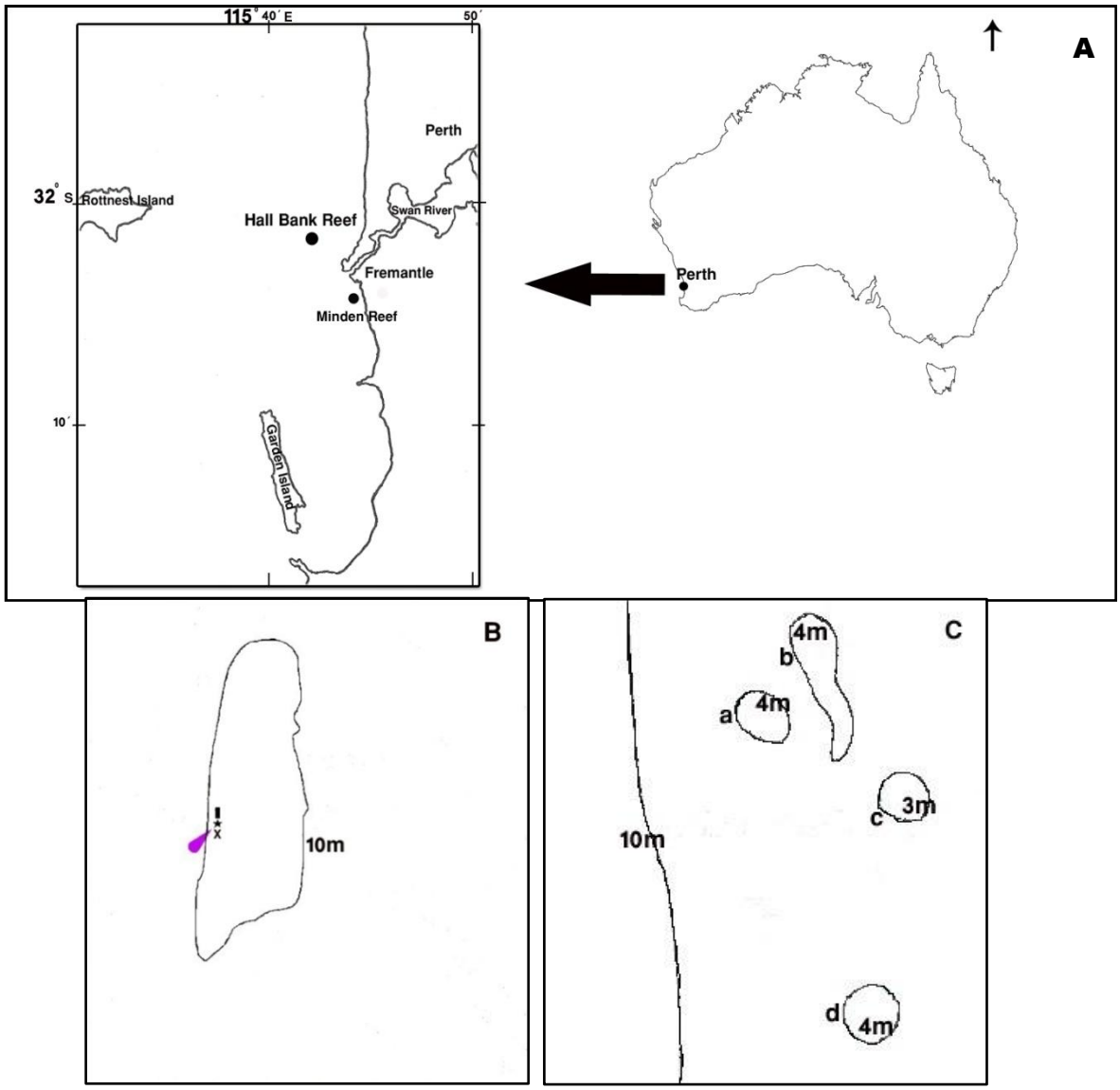

Figure 2.1 A-Map showing locations of the Hall Bank Reef and Minden Reef in Western Australia; B- diagrammatic representation of Hall Bank reef (x-channel marker); C-diagramatic representaion of Minden reef (composed of small reef patches a,b,c and d) depth marked in meters.

Hall Bank reef is located 3km north-west of Fremantle and is in close proximity to the Swan River outflow. The reef covers an area of ~2ha and depth ranges from 7-10m. The top of the reef is ~7m deep and the reef gradually drops down to the surrounding seagrass meadows at 15 m. The uniqueness of the reef is due to a lack of soft corals, lack of macroalgae and high coral cover (mean = 52.6 ± 4.65%), compared

to other high-latitude reefs (Thomson and Frisch 2010) (Figure 2.2 B/D). The most prominent feature of this reef is the high cover of favid corals and high density of the sea urchin *Centrostephanus tenuispinus.* Minden Reef is a macroalgae dominated reef located 5km south-east of Hall Bank reef. It is located approximately 1 km south of the Swan River mouth. The water at Minden Reef is often turbid due to the Swan River plume. The reef consists of small reef patches alternating with seagrass and sandy substrate; it is approximately 6m in depth. The reef is dominated by macroalgae, mainly large brown algae, and soft corals were abundant in this site as well. Unlike Hall Bank reef, this site exhibits typical characteristics of high latitude reefs (high abundance of macroalgae and soft corals and low coral cover).

2.2.2 Methods

The study was carried out from December 2015 to January 2017. Each site was visited once a month during the sampling period. Underwater surveys were carried out using belt transects and quadrats using SCUBA.

2.2.2.1 Estimating sea urchin density and size structure

Ten haphazard transects (20 m) were laid at each site. Eight transects were deployed 10m depth and two transects were deployed at 12m. Sea urchins were counted up to 0.5 m of each side of the transect lines (belt transect, 20 X 1 m). The density of urchins was calculated per square meter.

Considering the higher number of urchins at Hall Bank reef compared to Minden Reef, only urchins in two transects were used for size structure study at Hall Bank reef. Test diameters of all urchins from transect 1 and transect 10 were measured using Vernier calliper (± 0.01 mm) at the Hall Bank site. Urchins from all transects (10) at Minden Reef were used for this study. Urchins from the two study sites were categorized into size classes of 5 mm intervals.

Forty urchins collected for the reproductive biology study (Chapter 3) were used to calculate the Aristotle's Lantern Index (ALI).

$$Aristotle's\ Lantern\ Index = \frac{Lantern\ jaw\ height}{Test\ diameter} X\ 100$$

ALI of two study sites was compared with ANCOVA (with test diameter as a covariant).

2.2.2.2 Estimating substrate composition

The same transects laid for the urchin counts were used to analyse substrate composition. Quadrats (0.5 X 0.5 m) were placed at 5 m intervals along each transect (at

0, 5, 10, 15 and 20 m). Each quadrat was placed on the left side of the transect and photographed using an underwater camera (Olympus Tough TG3). Species present in quadrats were identified to class level. Coral species were identified up to species level. Fifty images per month per site were analysed using Coral Point Count with Excel extensions (V. 4, Kohler and Gill 2006). Substrate was categorised into live coral cover, dead coral cover, soft coral, sand and rubble, crustose coralline algae, turf, macroalgae, dead macroalgae, seagrass and colonial invertebrates for the analysis. Substrate composition data was imported into Primer v6 multivariate software (V.6, Primer-E Ltd.) for further analysis.

2.2.2.3 Diversity of other invertebrates and fish

The percentage cover of colonial attached forms of invertebrates was estimated using the same method in Section 2.2.2.2. Gastropods, bivalves and other echinoderms were counted in 20 x 1 m belt transects (10) in summer 2016 and winter 2016.The density of these organisms was calculated per square meter.

Four remote underwater cameras (GoPro) were used for the fish census. Cameras were deployed in four locations facing different directions at Hall Bank reef and Minden Reef in both summer and winter for 3 hours. Unbaited cameras were used so that it is unbiased towards the feeding habits of the fish. Visibility of the study sites was very low in both sites. Due to the high brown foliose algae cover at Minden Reef visibility of fish was low; therefore results from the recent survey of Richards et al. (2016) were used for Minden Reef diversity comparisons.

2.2.2.4 Analysis of water quality parameter

Seawater temperature was obtained using two in situ temperature loggers (HOBO-UA-002-64) at Hall Bank reef and Minden Reef (data logger at Hall Bank deployed by CSIRO).

2.2.2.5 Sea urchin exclusion experiment

Forty-five settlement plates (terracotta tiles, 9 X 9 cm) were fixed at Hall Bank reef in January 2017. Three categories of settlement plates were deployed: open, semi-enclosed and enclosed. Fifteen plates were fixed in each category. Plates in the semi-enclosed category were enclosed with a stainless steel cage, top open (1 X 1 cm mesh size) (Figure 2.2B). Thus, only fish feeding on the top had access and urchins were excluded. Plates in the enclosed category were caged from all sides (1 x 1 cm mesh size) (both fish and urchins excluded / Figure 2.2C). Out of 45 settlement plates 41

settlement plates were sampled after one month in February 2017. Four cages were washed away with water current and therefore excluded from analysis.

Figure 2.2 Plate showing the three categories of settlement plates at Hall Bank reef (A: Open plates, B: Semi-enclosed plates, C: Enclosed plates).

All plates were retrieved and transported to the laboratory in sealed bags with sea water for analysis. Each plate was overlaid with a 1 X 1 cm grid and observed under a dissecting microscope (Olympus Imaging, modelSZ61). Species present on each plate were identified up to genus level and the percent cover of each species estimated using the grid (9 X 9 cm).

2.2.3 Statistical Analysis

2.2.3.1 Monthly and seasonal differences in urchin densities

Differences in urchin densities between the two study sites, and monthly/seasonal variation within each study site were analysed using ANOVA in SPSS 24 software.

2.2.3.2 Substrate cover analysis

Similarities in substrate composition between two study sites were analysed using ANOSIM and permutational multivariate analysis of variance (PERMANOVA). Data were square root transformed, and Bray-Curtis similarity matrices were constructed separately for temporal data from Hall Bank Reef and Minden Reef. Seasonal data for Hall Bank and Minden Reef were used to represent the two-way crossed design of the site (2 levels) and season (2 levels), with each factor being fixed. PERMANOVA was tested on this design to check the possibilities of interactions and level of interaction these factors. Further, the matrix from temporal substrate composition data was subjected to one-way analysis of similarities (ANOSIM), with factors as month and season separately. R statistics and associated p values were used to interpret the output.

A SIMPER (similarity percentage) analysis was also used to determine the most abundant substrate component each month. Further, similarities in substrate composition between seasons and sites (Hall Bank and Minden Reef) were analyzed using Primer v6.

2.2.3.3 Correlation between urchin density and substrate cover

Scatter plots were constructed separately for each substrate category (coral cover, sand and rubble, crustose coralline algae, turf, macroalgae and seagrass) and urchin density. Pearson correlation was tested using SPSS 24 software.

2.2.3.4. Analysis of settlement plates

Similarities in substrate composition between settlement plate categories (enclosed, semi-enclosed and open) were analysed using ANOSIM and PERMANOVA. Data were square root transformed, and Bray-Curtis similarity matrices were constructed for substrate composition in each category. Further, the matrix from substrate composition data was subjected to one-way ANOSIM, with urchin exclusion as a factor. R statistics and associated p values were used to interpret the output. A SIMPER analysis was also used to determine most abundant substrate component in each category.

2.3 Results

2.3.1 *Centrostephanus tenuispinus* population parameters

2.3.1.1 Temporal variation of *C. tenuispinus* density Hall Bank Reef vs Minden Reef

The *Centrostephanus tenuispinus* population at Hall Bank reef (2.94 ± 0.14 individuals m^{-2}) had higher densities compared to the population at Minden Reef (0.14 ± 0.01 individuals m^{-2}) (monthly mean ± SE) ($p < 0.001$). Significant monthly variations in urchin densities were recorded at both sites (Hall Bank reef – $F_{(10,88)} = 3.671$, $p < 0.001$; Minden Reef – $F_{(8,102)} = 2.520$, $p = 0.017$). One-way ANOVA conducted for each site separately (season as factor) revealed there were no differences in seasonal densities of *C. tenuispinus* in two study sites (Hall Bank reef – $F_{(4,102)} = 2.340$, $p = 0.060$; Minden Reef – $F_{(4,88)} = 1.046$, $p = 0.389$).

Pair-wise comparisons revealed that urchin densities at Hall Bank are significantly lower in January 2016 (2.22 ± 0.30 individuals m^{-2}) and July 2016 (2.18 ± 0.21 individuals m^{-2}) than May 2016 (3.5 ± 0.25 individuals m^{-2}), August 2016 (3.61 ± 0.44 individuals m^{-2}) and November 2016 (6.63 ± 0.35 individuals m^{-2}) at Hall Bank reef. Mean urchin densities for summer 2016, autumn 2016, winter 2016 and spring

2016 were 2.70 ± 0.16, 3.20 ± 0.28, 2.82 ± 0.28 and 3.28 ± 0.23 individuals m^{-2} respectively (mean ± SE).

Sea urchin density of Minden Reef in December 2015 (0.25 ± 0.05 individuals m^{-2}) was significantly higher than densities in January 2016 (0.09 ± 0.04 individuals m^{-2}) and September 2016 (0.03 ± 0.02 individuals m^{-2}) (Figure 2.3). Mean density for summer 2016, autumn 2016, winter 2016 and spring 2016 were 0.16 ± 0.03, 0.13 ± 0.02, 0.16 ± 0.04 and 0.09 ± 0.03 individuals m^{-2} respectively.

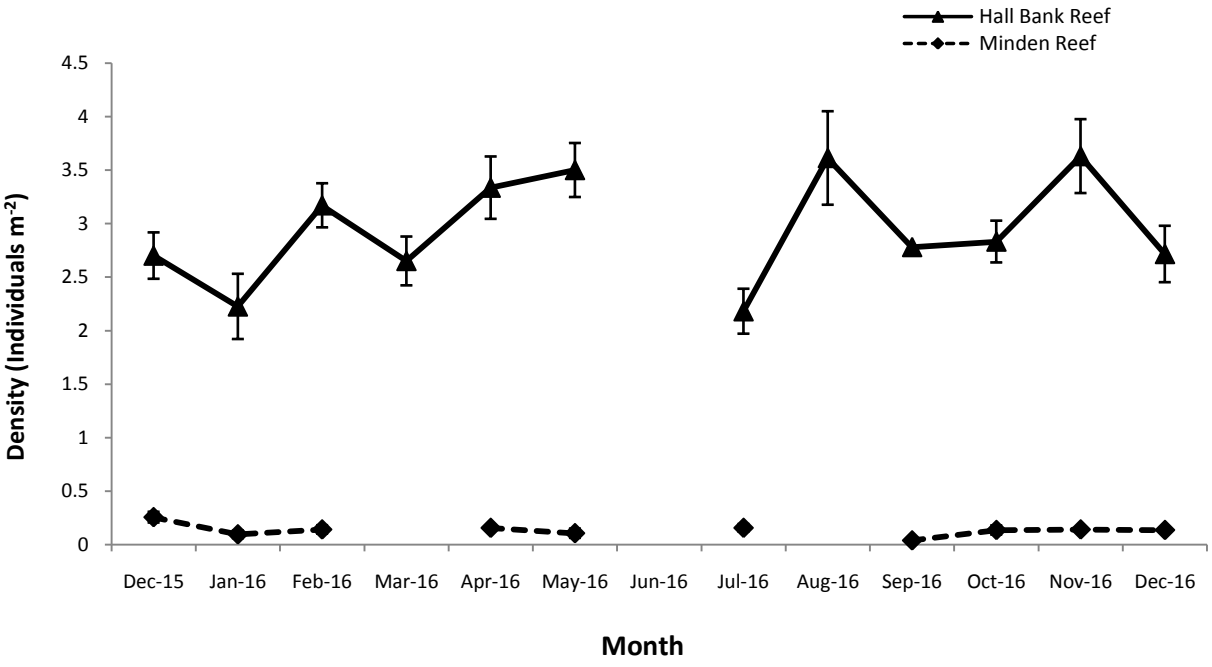

Figure 2.3. Monthly variation of *C. tenuispinus* density (individuals m^{-2}) (mean ± SE) at Hall Bank reef and Minden Reef.

2.3.1.2 Temporal variation of *C. tenuispinus* population size distribution

The population structure of *C. tenuispinus* in both sites is unimodal, with intermediate size urchins more dominant at Hall Bank reef and large sizes dominant at Minden Reef. Urchins at Minden Reef (83-118 mm) larger compared to urchins at Hall Bank reef (range 38-98 mm) ($p < 0.001$). Mean test diameters were 66.23 ± 0.24 mm and 100.69 ± 0.45 mm (monthly mean ± SE) at Hall Bank and Minden Reef respectively. The absence of small individuals was notable at Hall Bank reef (<38 mm) and Minden reef (<83 mm).

Urchins in the intermediate size category (60-65 mm) had the highest percentage frequency at Hall Bank (26.66 ± 1.93 %) (mean ± SE) (Figure 2.4). This size category was dominant from February 2016 – July 2016 and November 2016. Percent frequency was higher in size class of 70-75 mm in August (29.91 ± 1.49 %) (Figure 2.4). The

highest proportion of the *C. tenuispinus* population at Minden Reef was in the 100-105 mm size class, in December 2015, July 2016, September 2016 and November 2016 (Figure 2.4). February 2016- July 2016 samples mostly comprised of size class of 95-100mm (over 37 %). The largest class (115-120 mm) at Minden Reef was recorded only in December 2015. The smallest size class (80-85 mm) was recorded in both December 2015 and January 2016.

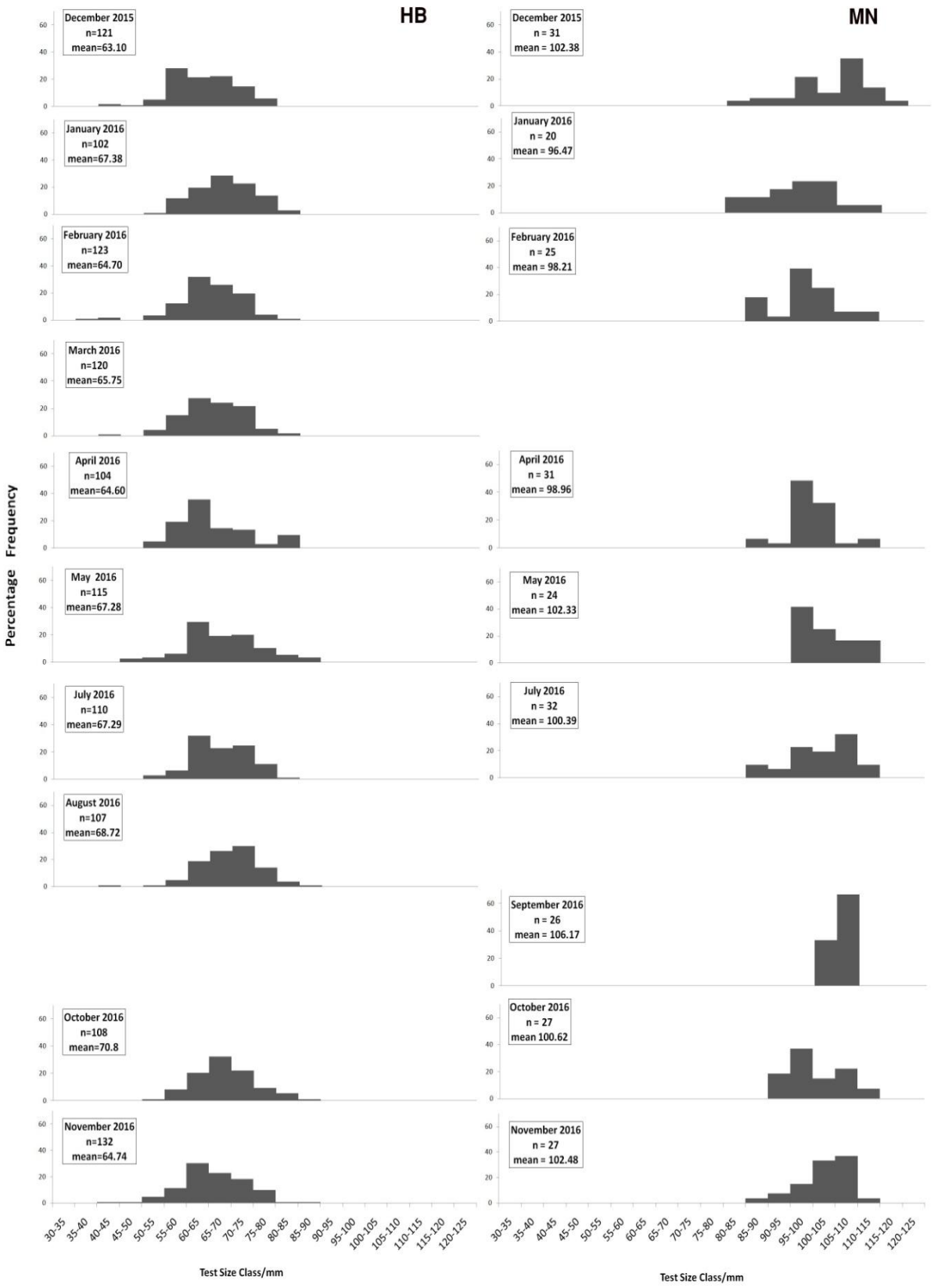

Figure 2.4 Monthly variation in test size distribution (test diameter/mm) in *C. tenuispinus* population at Hall Bank reef (HB) and Minden Reef (MN) from December 2015 – November 2016.

2.3.1.3 *Centrostephanus tenuispinus* population size distribution – Hall Bank reef vs Minden Reef

The majority of the population (71.19 %) was between 60-75 mm at Hall Bank reef. Size class < 60 mm made up16.72 % and size class >75 mm made up 12.08 % of the population at Hall Bank reef as well (Figure 2.5). On the other hand, percent frequency of urchins < 85 mm, 85-100 mm and > 100 mm were 8.26 %, 36.78 % and 54.96 % respectively at Minden reef. These results reflect the high abundance of intermediate-sized urchins at Hall Bank and high abundance of large urchins at Minden Reef.

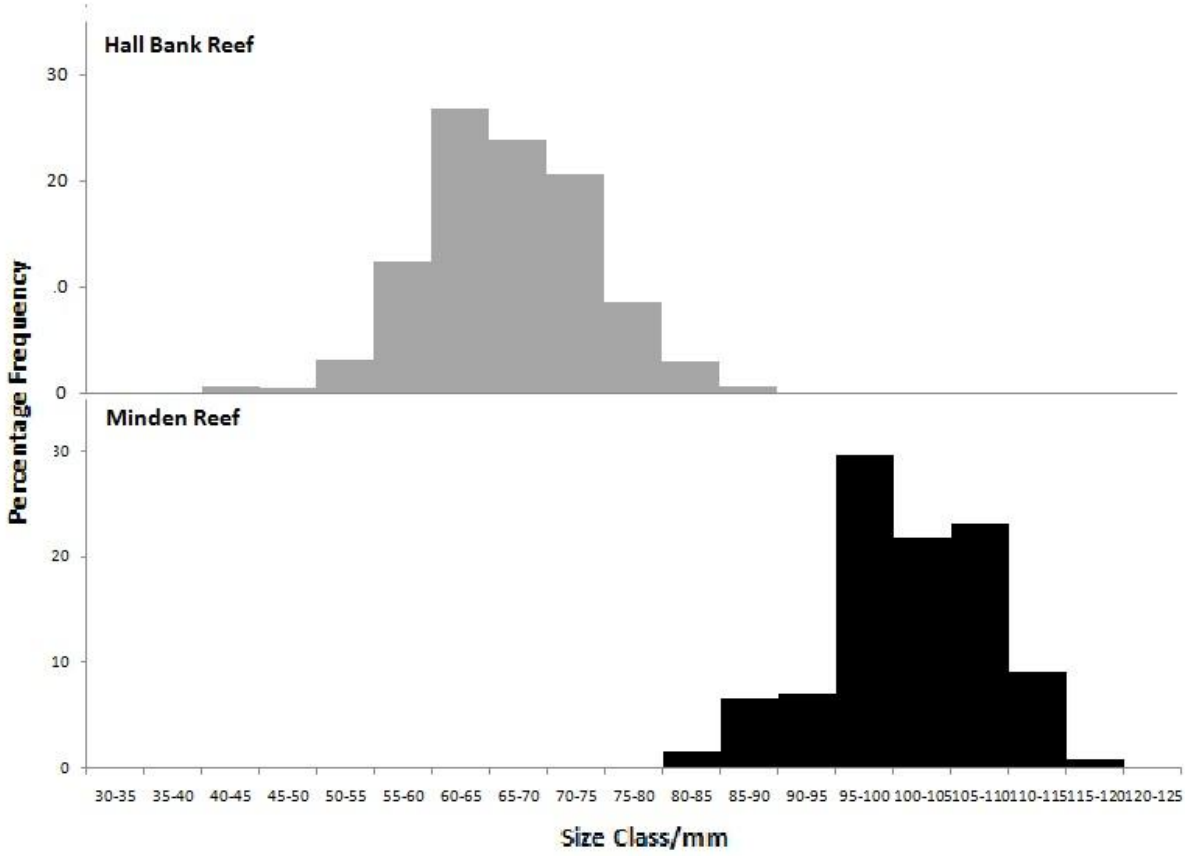

Figure 2.5 Population size distribution of the *C. tenuispinus* population at Hall Bank reef (gray bars) (n = 1142) and Minden Reef (black bars) (n = 242).

2.3.1.4 Morphometry of Aristotle's lantern in *C. tenuispinus* Hall Bank reef vs Minden Reef

The Aristotle's lanterns of *C. tenuispinus* were heavier and larger at Minden Reef compared to urchins from Hall Bank reef. Yet, Minden Reef urchins had a relatively lower Aristotle's Lantern Index (ALI) (winter- 28.33 ± 1.46 %; summer - 29.56 ± 1.44 %) than urchins from Hall Bank reef ($F_{(1,40)}$ = 15.537, $p < 0.001$) (Figure

2.10). The ALI of urchins from Hall Bank in summer and winter were 37.14 ± 1.35 % and 36.07 ± 3.15 % (mean ± SD) respectively (Table 2.2 / Figure 2.6). No differences were observed in the ALI with respect to the season in both sites ($F_{(1,40)} = 12.045$, $p = 0.096$).

Table 2.2 Test weights, test diameters and Aristotle's lantern morphometrics (mean ± SE) of *C. tenuispinus* at Hall Bank reef and Minden Reef in summer and winter (n = 40).

	Hall Bank reef		Minden Reef	
	Summer	Winter	Summer	Winter
Total weight (g)	129.38±5.02	137.52±7.89	247.78±14.26	315.69 ±25.16
Test diameter (mm)	68.29±1.61	68.47±1.31	95.05±1.76	98.94±2.35
Test height (mm)	27.415±0.62	29.61±0.48	42.62±1.53	44.97±1.49
Aristotle's lantern weight(g)	8.59±0.61	8.85±0.66	12.2014±0.77	14.26±1.13
Jaw height (mm)	25.34±0.81	24.73±0.68	28.12±0.75	28±0.67
Lantern Diameter (mm)	24.24±0.54	23.76±0.79	26.85±0.81	27.80±0.66
ALI (%)	37.14±1.35	36.07±3.15	29.56 ± 1.44	28.33±1.46

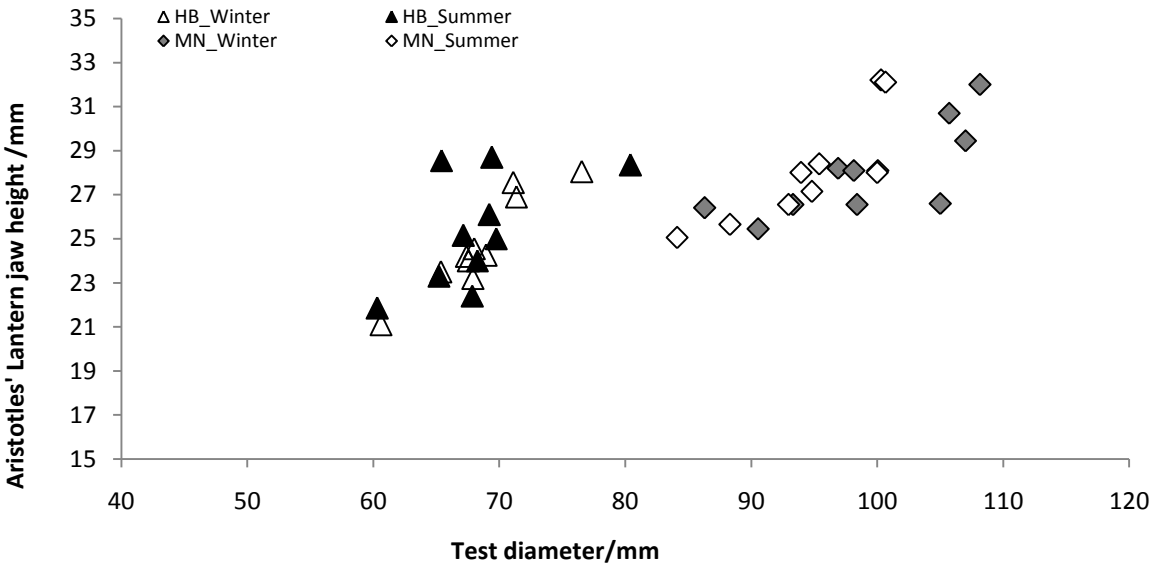

Figure 2.6 Relationship between urchin test diameter (mm) and Aristotle's lantern height (mm) at Hall Bank reef (winter and summer represented by black and white triangles) and Minden Reef (winter and summer represented by gray and white diamonds)(n = 40).

2.3.2 Temporal variation in substrate cover

2.3.2.1 Monthly variation in substrate cover; Hall Bank reef vs Minden Reef

The substrate composition of Hall Bank reef was significantly different from Minden Reef (ANOSIM one-way R = 0.954, p = 0.1%; PERMANOVA pseudo-F = 386.2, p = 0.001) (Figure 2.7). The main contributors to substrate composition at Hall Bank reef were turf algae (35.81 %) and crustose coralline algae (14.62 %), while seagrass (27.20 %) and macroalgae (22.93 %) contributed to the substrate composition of Minden Reef. Sand, pavement and rubble were also important contributors to

substrate composition (combined contribution: Hall Bank = 23.97 %; Minden Reef = 19.99 %). At Hall Bank reef, this substrate category was mainly rubble (17.30 ± 1.41 %) and at Minden Reef it was sand (14.30 ± 1.65 %) (Figures 2.8, 2.9). Coral, turf algae (filamentous microalgae), colonial invertebrates, crustose coralline algae, sand/sediment, pavement and rubble were major substrate categories recorded in both study sites. Main contributors for the substrate composition at Minden Reef, were macroalgae and seagrass.

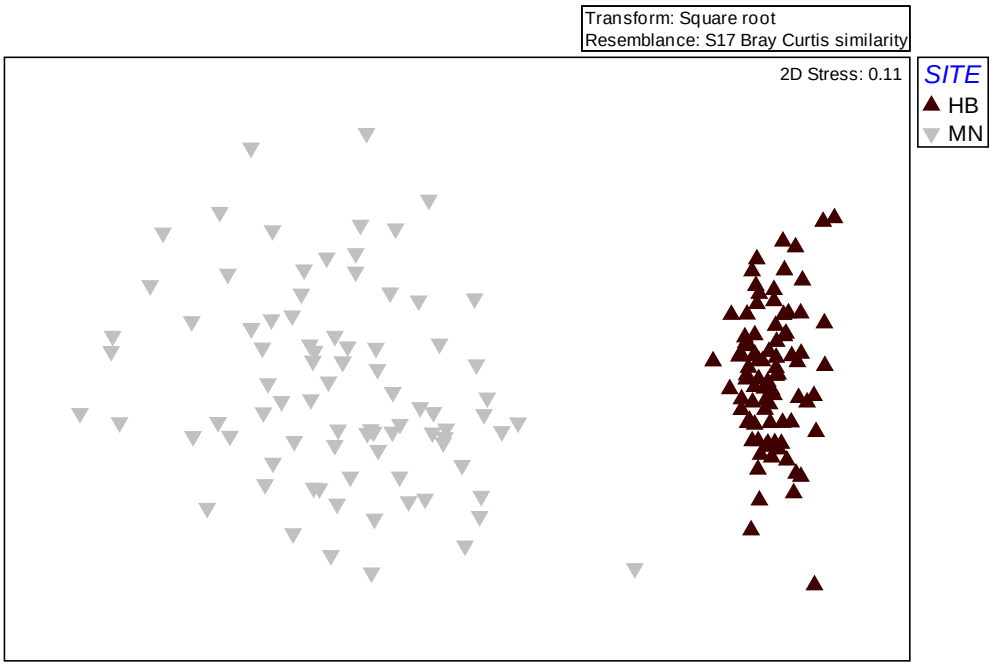

Figure 2.7 nMDS ordination plots, matrices constructed from Bray-Curtis matrices of the percentage cover of the substrate categories at Hall Bank reef (HB) and Minden Reef (MN).

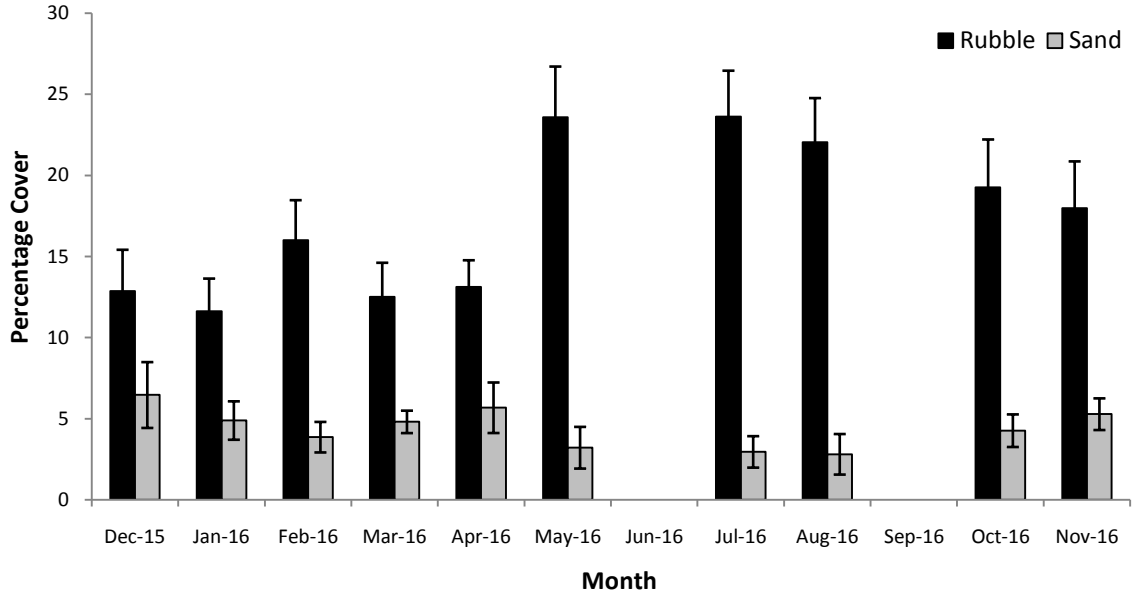

Figure 2.8 Monthly variation of sand and rubble percentage at Hall Bank reef (mean ± SE).

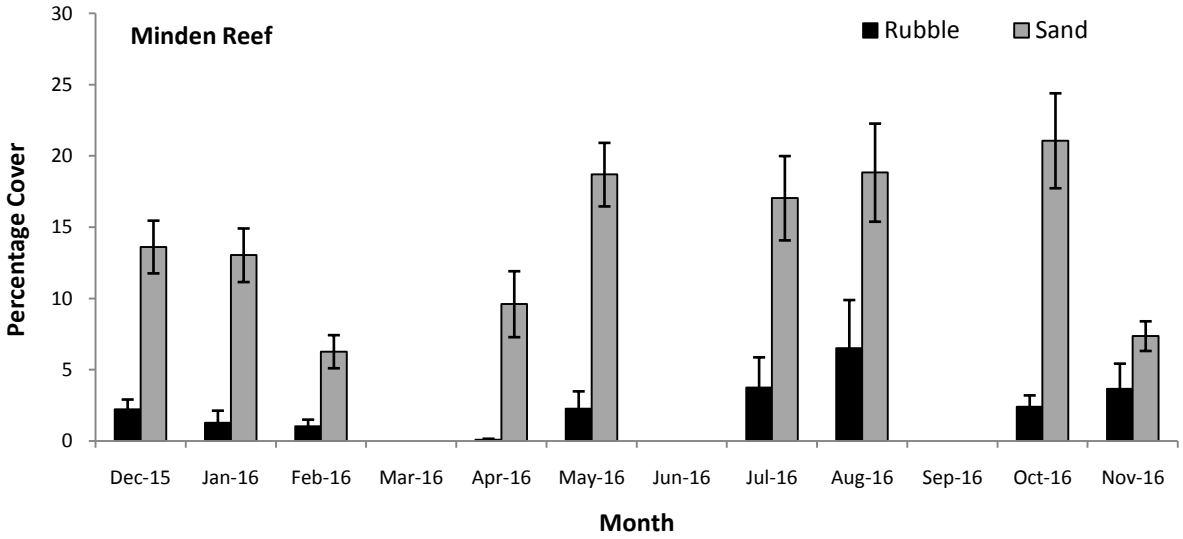

Figure 2.9 Monthly variation of sand and rubble percentage at Minden Reef (mean ± SE).

Mean monthly percentage of turf algae, coral, coralline algae, colonial invertebrates and sand and rubble were 44.27 ± 2.83 %, 12.61 ± 2.04 %, 9.8 ± 1.08 %, 10.29 ± 1.28 % and 21.69 ± 1.19 % (mean ± SE) respectively for Hall Bank reef. Mean monthly percentage of turf algae, coral, coralline algae, colonial invertebrates, macroalgae, seagrass, soft corals and sand and rubble were 12.24 ± 2.75 %, 3.14 ± 0.65 %, 0.43 ± 0.11 %, and 6.59 ± 1.01 %, 25.07 ± 3.88 %, 31.70 ± 2.90 %, 0.04 ± 0.02 % and 16.65 ± 2.06 % respectively for Minden Reef.

Lower global R values (one-way ANOSIM) for both monthly (R = 0.148, p = 0.1 %) and seasonal (R = 0.135, p = 0.1 %) substrate compositions at Hall Bank reef

reflect similarities in substrate composition in this site all year (Figure 2.10). Pair-wise comparisons showed higher R values between December and February (R = 0.766, p = 0.2 %), and October (R = 0.652, p = 0.2 %). This could be due to a high contribution from scleractinian coral in December. Pair-wise comparisons between seasons showed differences in substrate composition between seasons are very low (Figure 2.11). Turf algae and sand, pavement and rubble contribute equally for substrate cover year round at Hall Bank reef (SIMPER). This explains the low separation between months/ season at Hall Bank reef.

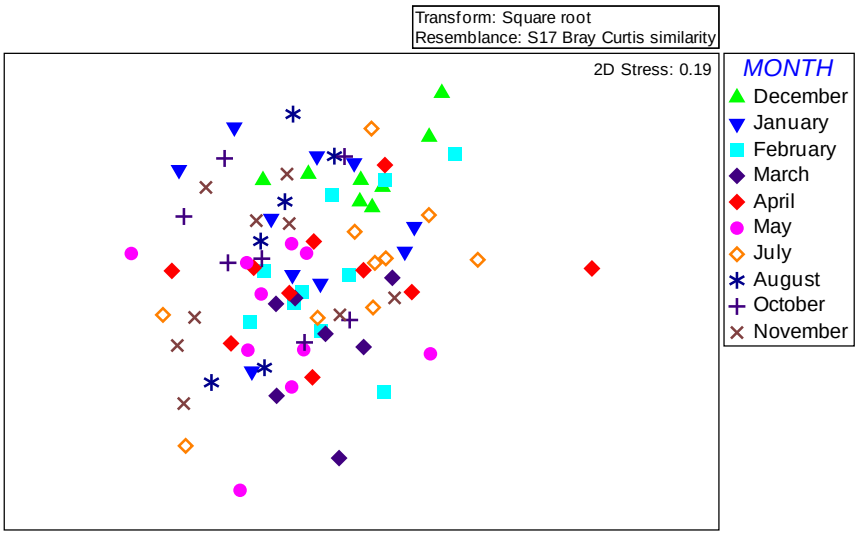

Figure 2.10 nMDS ordination plots, matrices constructed from Bray-Curtis matrices of the percentage mean monthly cover of various substrate categories contributing for substrate composition at Hall Bank reef (December 2015 to November 2016 represented by numbers 1-12).

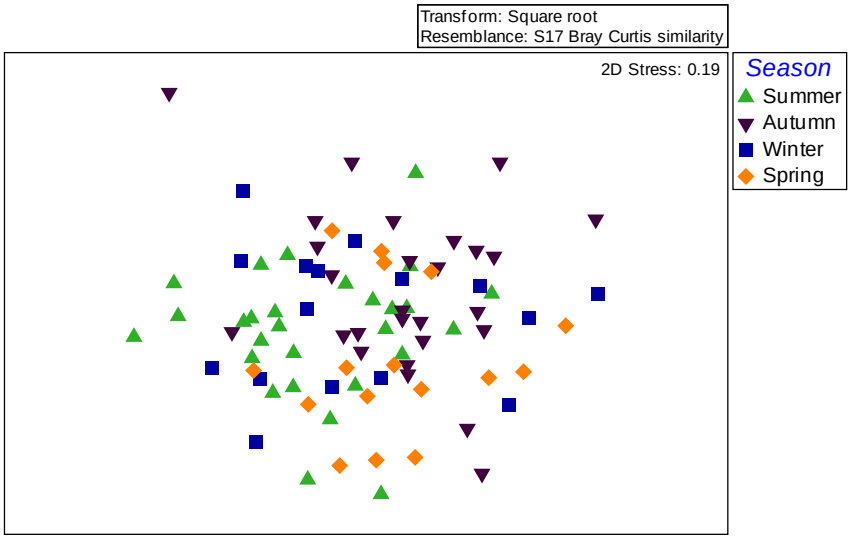

Figure 2.11 nMDS ordination plots, matrices constructed from Bray-Curtis matrices of the percentage mean seasonal cover of various substrate categories contributing for substrate composition at Hall Bank reef.

One-way ANOSIM revealed significant differences in substrate composition between months at Minden Reef (R = 0.519, p = 0.15 %) (Figure 2.12). These differences were mainly between December-May and June-November. Pair-wise comparisons revealed less separation between September, October and November and substrate cover from July was similar to these months as well. Turf algae, sand and sponges were the main contributors in December and January. *Dictyopteris* sp. had the highest contribution (21.76 %) for February. A one-way ANOSIM with the season as a factor supported this outcome as well (Figure 2.13). Substrate composition was similar in summer and autumn (R = 0.093, p = 2.6 %). Substrate composition of both these seasons was significantly different from spring. Further, substrate cover was similar in winter and spring (R = -0.062, p = 74.5 %). SIMPER revealed that this similarity is mainly due to the high contribution of *Sargassum* sp. in the two seasons (winter = 23.25 %; spring = 33.68 %).

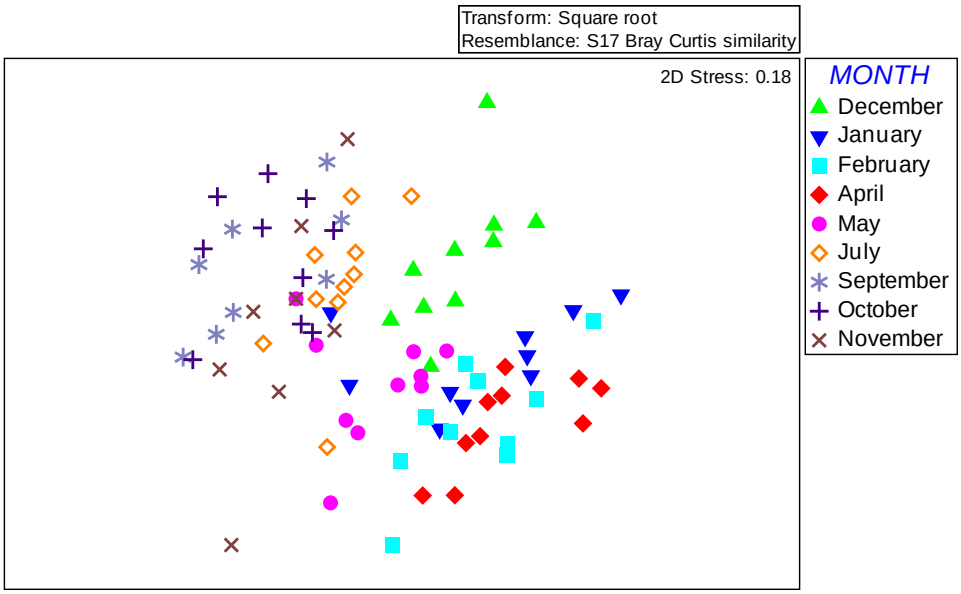

Figure 2.12 nMDS ordination plots, matrices constructed from Bray-Curtis matrices of the percentage mean monthly cover of various substrate categories contributing for substrate composition at Minden Reef.

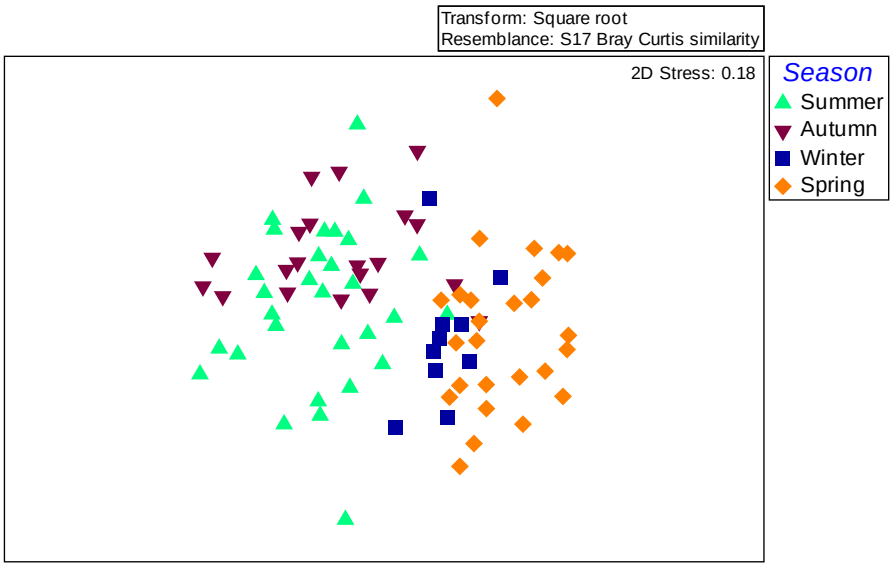

Figure 2.13 nMDS ordination plots, matrices constructed from Bray-Curtis matrices of the percentage mean seasonal cover of various substrate categories contributing for substrate composition at Minden Reef.

Four species of seagrass were recorded from Minden Reef; namely, *Posidonia* sp. *Halophila* sp., *Amphibolis* sp. and *Syringodium isoetifolium* (Figure 2.14). *Posidonia* sp. (20.73 ± 2.38%) (seasonal mean ± SE) was the most abundant seagrass species at Minden Reef. This species was highly abundant in autumn (range 10.17 - 19.05 %) and spring (range 20.21 - 35.57 %). The highest percentage recorded in individual transect for *Posidonia* sp. was 66.4% in October 2016. *Halophila* sp. (9.69 ± 2.37%) (seasonal mean ± SE) was mostly abundant in summer (range 5.68 - 14.38 %) and autumn (range- 14.13 - 15.16 %).

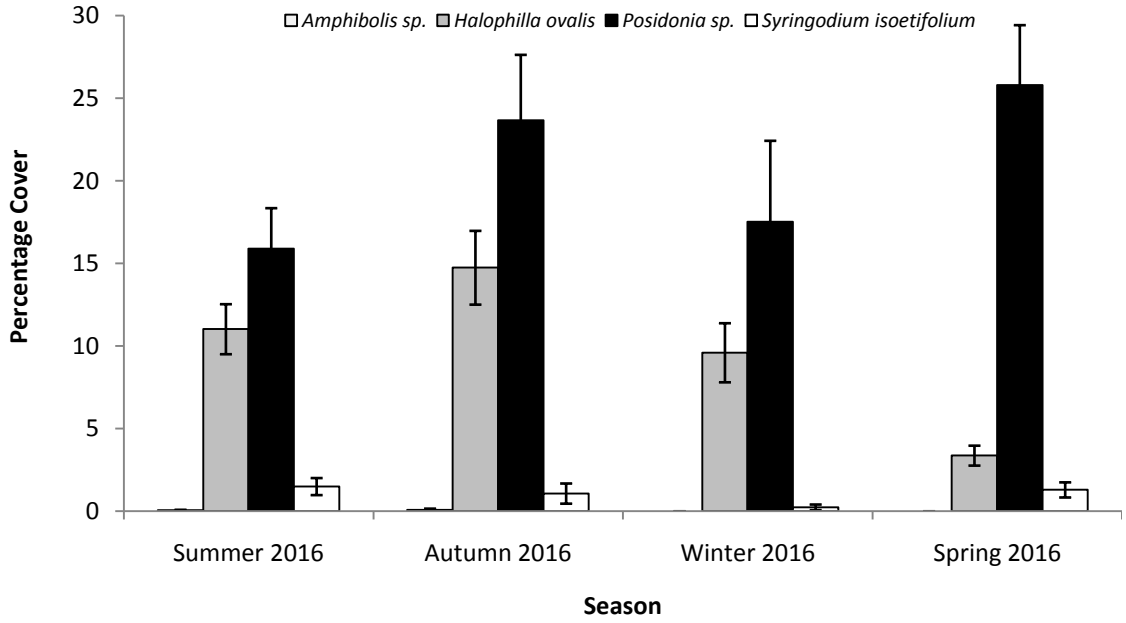

Figure 2.14 Seasonal variation of percentage seagrass cover at Minden Reef (mean ± SE) (summer 2016 - spring 2016).

Eleven species of algae were identified form Minden Reef (Figure 2.15). Seasonal variations in brown foliose algae were also observed. *Sargassum* sp. was the most abundant brown foliose algae at Minden Reef. *Sargassum* sp. was highly abundant in winter 2016 (30.72 %) and spring 2016 (33.72 %) while *Dictyopteris sp.* was mostly abundant in autumn 2016 (12.60 %). The green algae *Codium* and *Halimeda* sp. were also recorded in summer 2016 and autumn 2016.

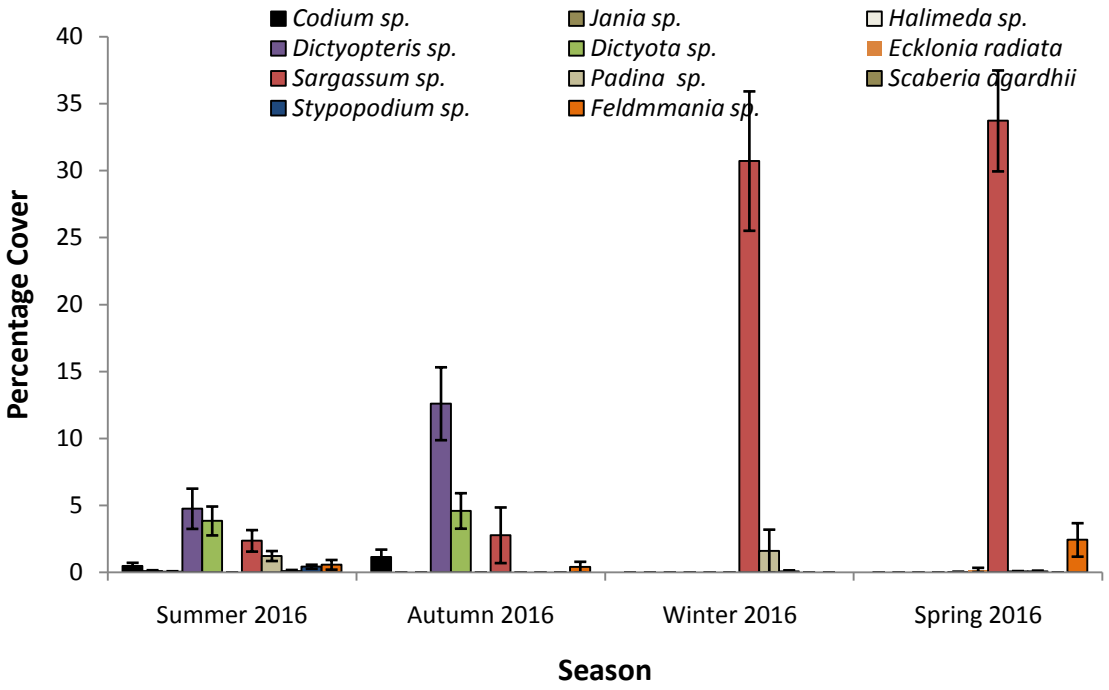

Figure 2.15 Seasonal variation of percentage macroalgae cover at Minden Reef (mean ± SE) (summer 2016 - spring 2016).

Percentages for crustose coralline algae were higher at Hall Bank Reef (mean 9.8 ± 1.08 %) than Minden Reef (0.43 ± 0.11 %) (Figure 2.16). Turf algae were the most abundant substrate category year-round at Hall Bank (individual percentages for transects, 1.60 - 67.20 %). The proportion of turf algae at Minden Reef (12.24 ± 2.75 %) was very low compared to Hall Bank reef.

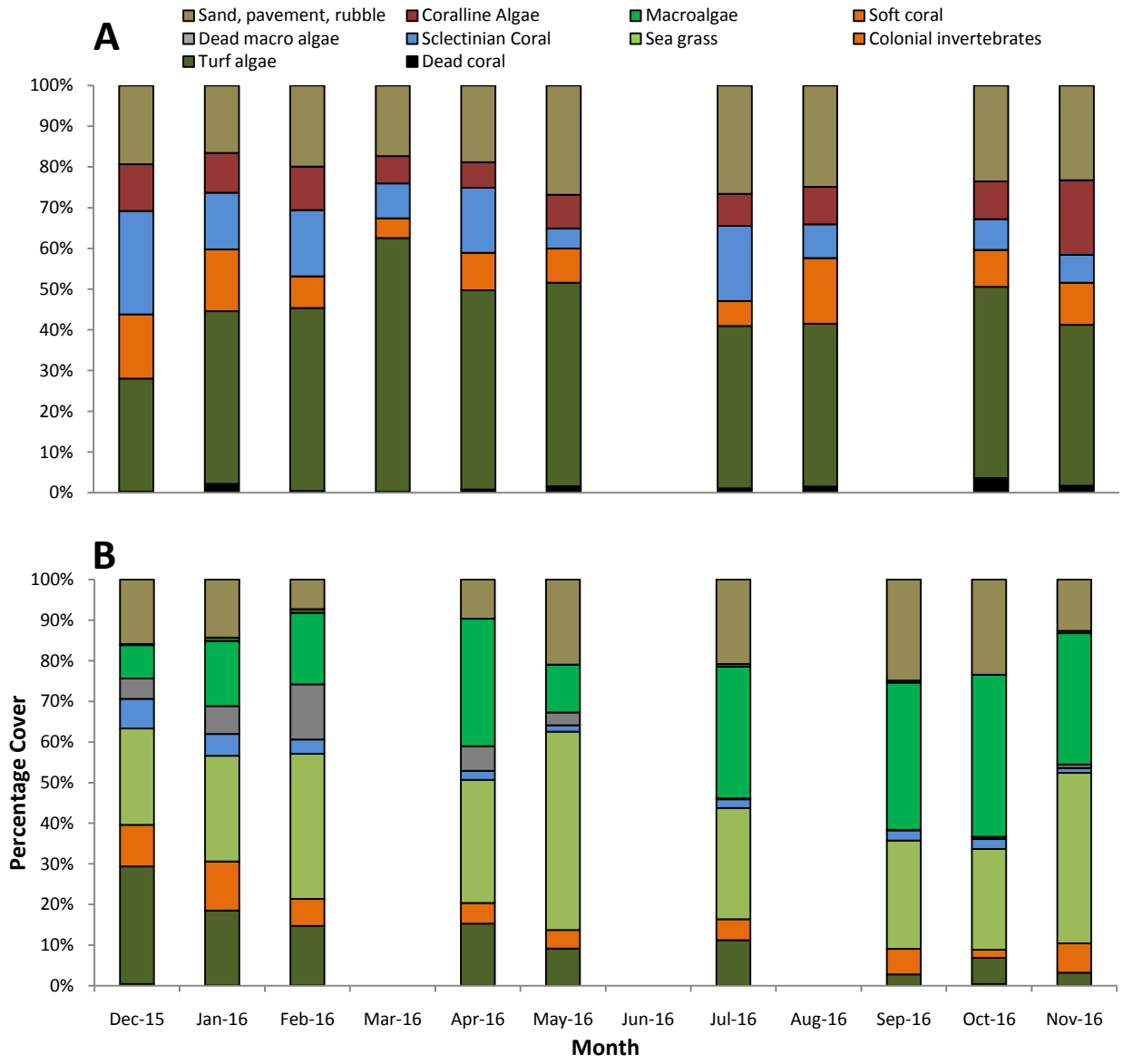

Figure 2.16 Monthly variation of substrate composition at Hall Bank reef (A) and Minden Reef (B).

Turf algae were the most abundant substrate category at Hall Bank reef. Mean turf cover for summer 2016, autumn 2016, winter 2016, spring 2016 and summer 2017 was 39.13 ± 2.58 %, 52.78 ± 2.13 %, 39.93 ± 2.46 %, and 42.34 ± 2.09 % and 35.84 ± 3.29 % (mean ± SE) respectively (Figure 2.17/2.18). Highest mean percentage macroalgae recorded for winter (32.40 ± 4.30 %) and spring (36.40 ± 3.39 %) at Minden Reef. Coral cover was highest in summer (summer 2016 - 18.04 ± 2.44 %; summer 2017- 21.39 ± 3.41 %). The sand percentage was highest in winter (25.94 ± 2.24 %). Colonial invertebrates which mostly consisted of sponges ranged from 7.79 ± 1.37 to 12.70 ± 1.55 %.

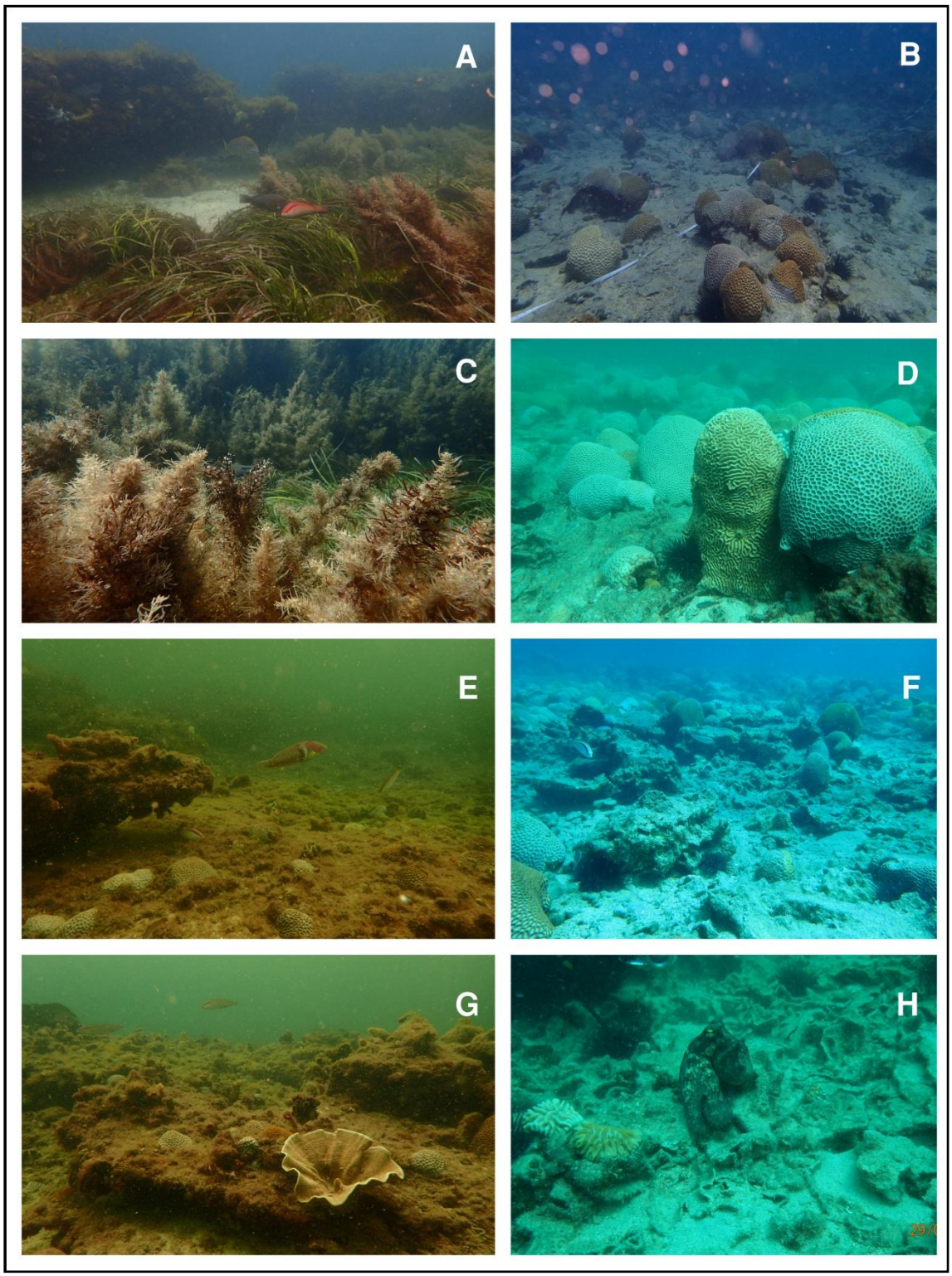

Figure 2.17 Photographs representing nature of substrate cover at Minden Reef (A/C: Minden Reef winter, E/G: Minden Reef summer, B/D coral dominated area at Hall Bank reef, F/H Turf algae dominated area at Hall Bank reef).

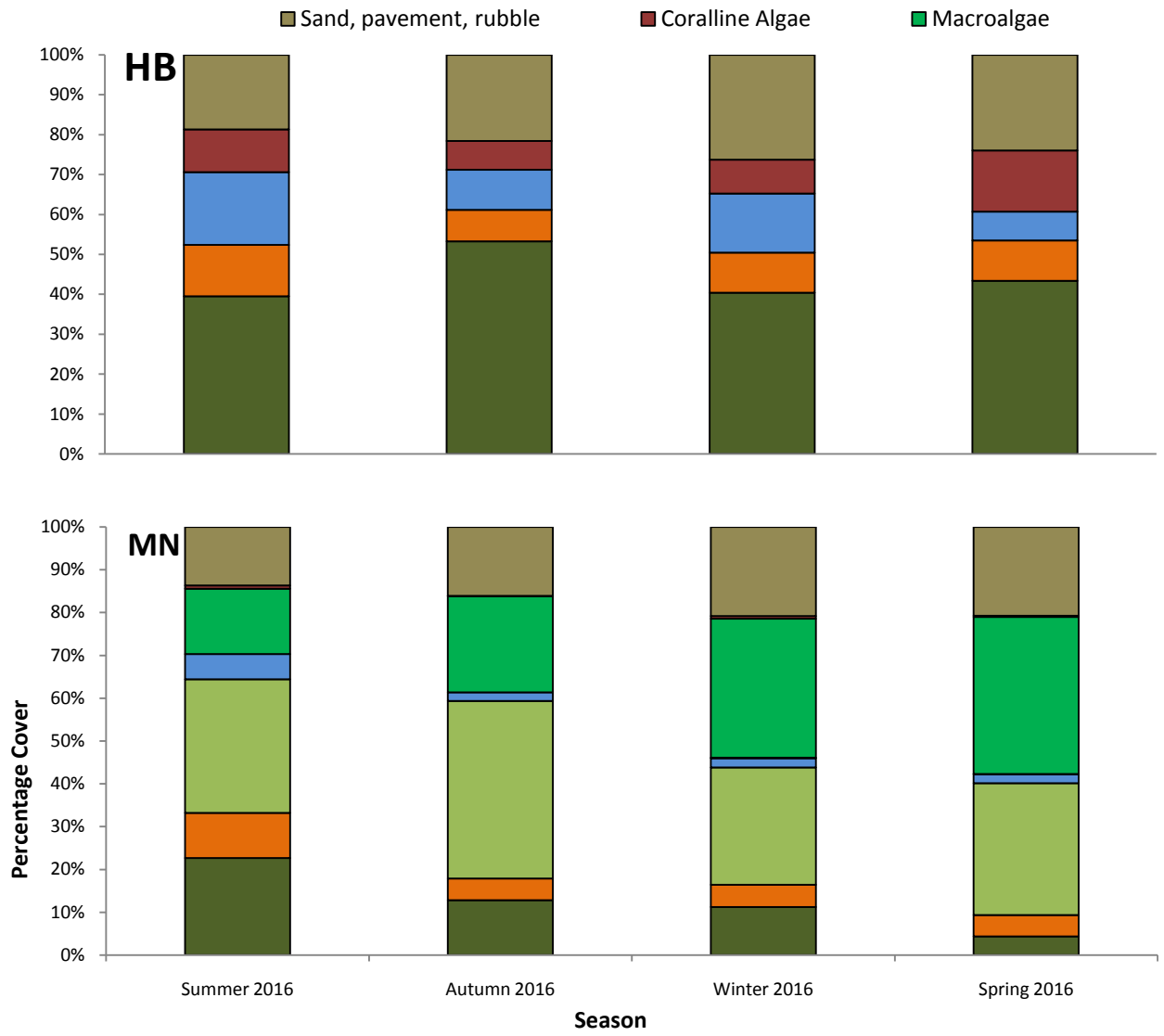

Figure 2.18 Seasonal variation of substrate composition at Hall Bank reef (HB) and Minden Reef (MN) (mean ± SE).

Individual coral cover ranged from 0.00 - 52.80 %. Highest coral cover recorded for individual transect was 52.80 % at Hall Bank while it was 44.47 % at Minden Reef. Out of a total of 101 transects analysed scleractinian corals were absent in 9 transects at Hall Bank reef. Mean coral cover at Hall Bank (12.61 ± 2.04 %) was four times higher than that of Minden Reef (3.14 ± 0.65 %).

Twelve species of scleractinian corals were observed at Hall Bank reef (Table 2.3). *Pocillopora damicornis* and *Goniopora* sp. were not encountered in transects, and thus not included in calculations. *Favia favus, Favites abdita, Coelastrea aspera, Goniastrea palauensis and Paragoniastrea australensis* were encountered at Hall Bank reef most part of the year. The most abundant species was *Goniastrea palauensis* (Figure 2.19).

Similarly, 12 species of scleractinian corals were recorded at Minden Reef; however, the composition was different. *Favites abdita*, *Favia rotundula Coelastrea aspera*, *and Plesiastrea versipora* and *Paragoniastrea australensis* were encountered year-round at Minden Reef. *Goniastrea palauensis* (Figure 2.19). *Coelastrea aspera* was the most abundant coral species at Minden Reef.

Table 2.3 Family and Species names of scleractinian coral species recorded at Hall Bank Reef and Minden Reef (presence indicated by "√").

Family	Species	Hall Bank Reef	Minden Reef
Faviidae	*Favia favus*	√	√
	Favia rotundula		√
	Favia pallida		√
	Favites abdita	√	√
	Coelastrea aspera	√	√
	Paragoniastrea australensis	√	√
	Goniastrea palauensis	√	
	Plesiastrea versipora	√	√
Acroporidae	*Montipora molis*	√	
Mussidae	*Symphyllia wilsoni*	√	
Dendrophylliidae	*Turbinaria frondens*	√	√
	Turbinaria peltata		√
	Turbinaria mesenterina	√	√
Pocilloporidae	*Pocillopora damicornis*	√	√
Siderastreidae	*Coscinaraea mcneili*		√
Poritidae	*Goniopora* sp.	√	

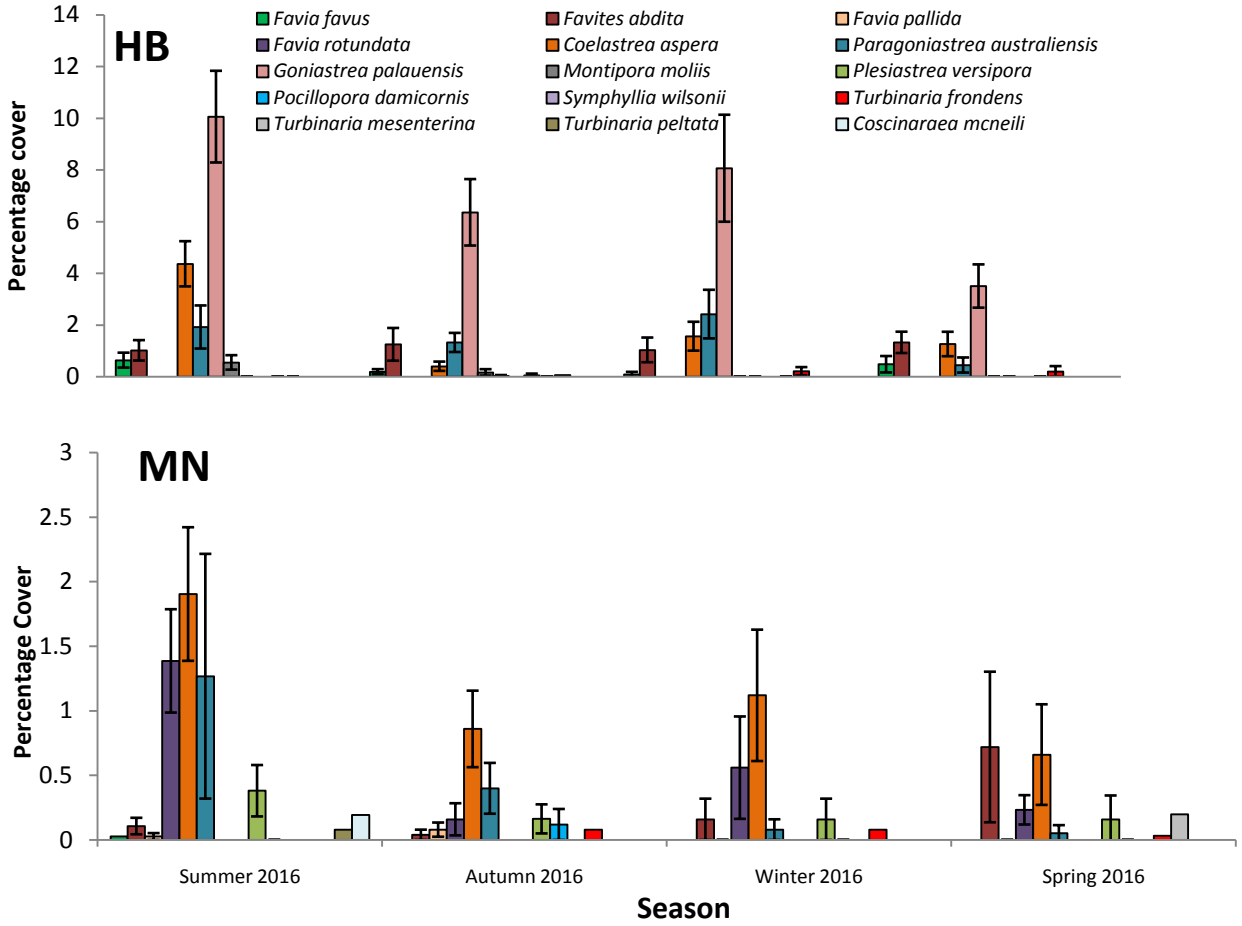

Figure 2.19 Seasonal variation of percentage cover of scleractinian coral species recorded at Hall Bank reef (HB) and Minden Reed (MN) from summer 2016 – Spring 2016 (mean ± SE).

The invertebrate community at Hall Bank reef is composed of mainly sea urchins (see 2.3.1), other echinoderms (star fish and sea cucumbers), molluscs (gastropods, bivalves and cephalopods), crustaceans (lobsters), polychaetes, sponges, ascidians and bryozoans. The main sea urchin species recorded at Hall Bank reef was *Centrostephanus tenuispinus*. One individual of sea urchin *Phyllacanthus irregularis* was recorded once. In contrast, *Centrostephanus tenuispinus* co-exists with *P. irregularis* (0.056 ± 0.011 individuals m^{-2}) and *Heliocidaris erythrogramma* (0.015 ± 0.006 individuals m^{-2}) (mean ± SE) at Minden Reef (Figure 2.20). The highest densities of *P. irregularis* (0.12 ± 0.018 individuals m^{-2}) and *Heliocidaris erythrogramma* (0.06 ± 0.03 individuals m^{-2}) were observed in October 2016.

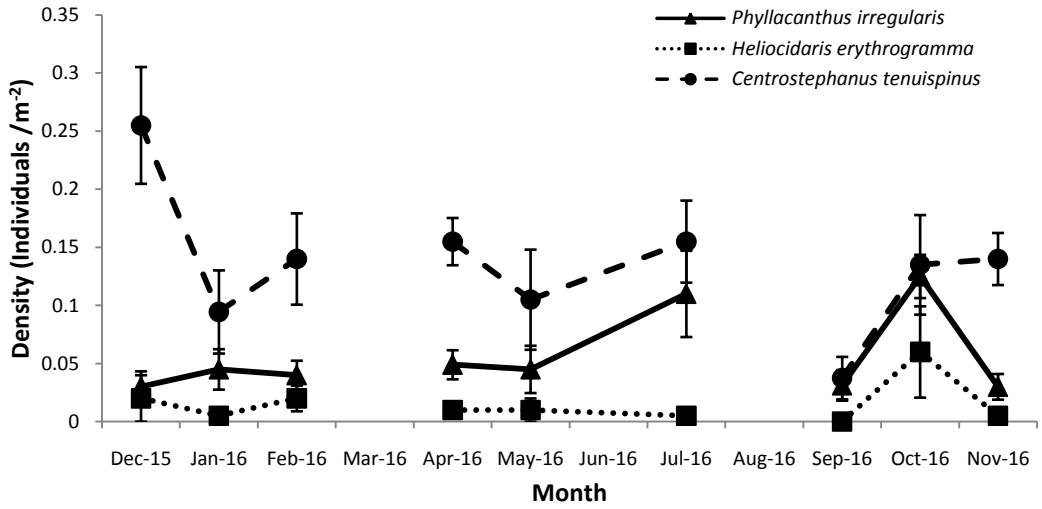

Figure 2.20 Temporal variation of sea urchins density (individual m-2) (mean ± SE) at Minden Reef (*C. tenuispinus*, *P. irregularis* and *H. Erythrogramma* represented by dashed line, line and dotted line respectively).

Filter-feeding polychaetes (e.g. sabellids) and oysters were also frequent at Hall Bank reef. Gastropods at Hall Bank reef were represented by shelled grazers and nudibranchs. Six species of nudibranchs (two species in genus *Chromodoris* sp., *Chromodoris elisabethina*, *Tambja morosa*, *Glossodoris undaurum* and *Halgerda punctata*) were recorded at Hall Bank reef. The most common gastropod was *Trochus* sp. and mean density of *Trochus* sp. was higher in summer 2016 (1.6 ± 0.17 individuals m^{-2}) than winter 2016 (0.98 ± 0.09 individuals m^{-2}).

Sponges were highly abundant compared to other invertebrates at both Hall Bank reef (9.43 ± 1.01 %) and Minden Reef (6.43 ± 0.99 %) (Figure 2.21). The highest percentages of sponges were recorded in August 2016 for Hall Bank reef and January 2016 for Minden Reef.

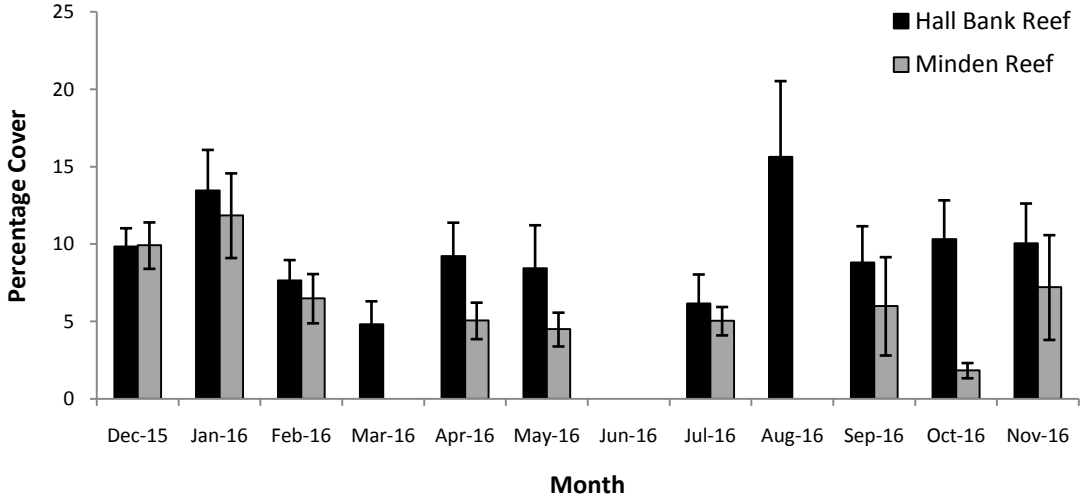

Figure 2.21 Monthly variation of percentage cover of sponges at Hall Bank reef (black bars) and Minden Reef (gray bars) (mean ± SE).

Bryozoan and ascidian colonies were only present in August and September 2016 and were represented by few species at Hall Bank reef. But the groups were highly diverse and abundant year-round (bryozoans 0.07 ± 0.03 %; ascidians 0.08 ± 0.03 %) (mean ± SE) at Minden Reef.

None of the substrate categories showed significant correlations with *C. tenuispinus* density at Hall Bank reef. Turf algae cover at Minden Reef showed significant negative correlation (Pearson correlation = 0.718, p = 0.029) with *C. tenuispinus* density.

The remote underwater visual census revealed the presence of 17 fish species at Hall Bank reef in contrast to the 46 species of fish at Minden Reef (Richards et al. 2016) (Table 2.4).

Table 2.4 Family, Species and common names of fish species recorded at Hall Bank reef.

Family	Species	Common name
Carangidae	*Pseudocaranx dentex*	Silver trevally
	Pseudocaranx wrighti	Sand trevally
Chaetodontidae	*Chelmonops sp.*	Western talma
Cheilodactylidae	*Cheilodactylus gibbosus*	Crested morwong
Labridae	*Dotalabrus aurantiacus*	Castelnau's wrasse
	Choerodon rubescens	Baldchin grouper
	Coris auricularis	Western king wrasse
Monacanthidae	*Scobinichthys granulatus*	Rough leatherjacket
Nemipteridae	*Pentapodus vitta*	West Australian Butterfish
Orectolobidae	*Orectolobus sp.*	Western wobbegong
Pempheridae	*Pempherisklun zingeri*	Rough bullseye
Pomacentridae	*Chromis westaustralis*	West Australian puller
	Abudefduf sordidus	Black spot sergeant major
	Pomacentrus milleri	Millers damselfish
	Pseudolabrus parilus	Brown spotted wrasse
	Parma occidentalis	Western scalyfin
	Stegastes obreptus	Western gregory

2.3.3 Impact of sea urchins grazing on substrate composition - sea urchin exclusion experiment

There were significant differences between substrate composition of open, semi-enclosed and enclosed settlement plates (PERMANOVA, pseudo F = 10.564, p = 0.001; ANOSIM, R = 0.589, p = 0.1 %) (Figure 2.22). Pair-wise tests revealed that there were significant differences in species composition between enclosed and open (R = 0.894, p = 0.8 %), and semi-enclosed and open treatments (R = 0.75, p = 2.4 %). No significant difference was recorded between the enclosed and semi-enclosed categories (R = 0.281, p = 11.4 %).

The number of substrate categories in enclosed plates, semi-enclosed plates and open plates was 8, 7 and 11 respectively. Open plates had the highest diversity of substrate settlement categories (Figure 2.23).

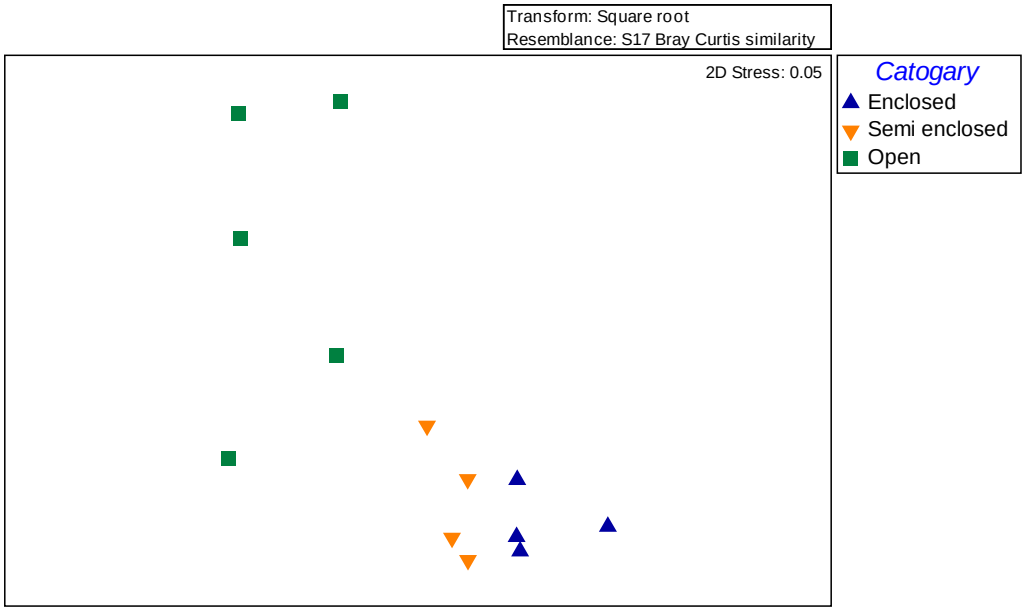

Figure 2.22 nMDS ordination plot, constructed from a Bray-Curtis matrix of the percentage cover of various substrate categories contributing for substrate composition in three categories of settlement plates.

Figure 2.23 Photographs showing settlements of algae and invertebrates on experimental plates one month after placement (A- open plates, B- semi-enclosed plates, C- enclosed plates).

SIMPER analysis confirmed that *Feldmannia mitchelliae*, polychaetes and *Cladophora* sp. highly contributed to similarities between enclosed and semi-enclosed plates (*Feldmmania mitchelliae* : enclosed = 47.10 ± 4.39 %, semi enclosed = 31.15 ± 5.39 % / polychaetes: enclosed = 12.92 ± 1.70 %; semi-enclosed = 13.01 ± 5.11 % and *Cladophora* sp.: enclosed = 7.69 ± 1.65 %; semi-enclosed = 9.21 ± 1.91 %) (Figure 2.24).

The SIMPER analysis indicated crustose coralline algae was the highest contributor for proportions (26.81 %) in open plates. Percentage cover of crustose coralline algae was lower in percentage in both enclosed and semi-enclosed plates (1.23 ± 0.63 % and 2.94 ± 1.11 %).

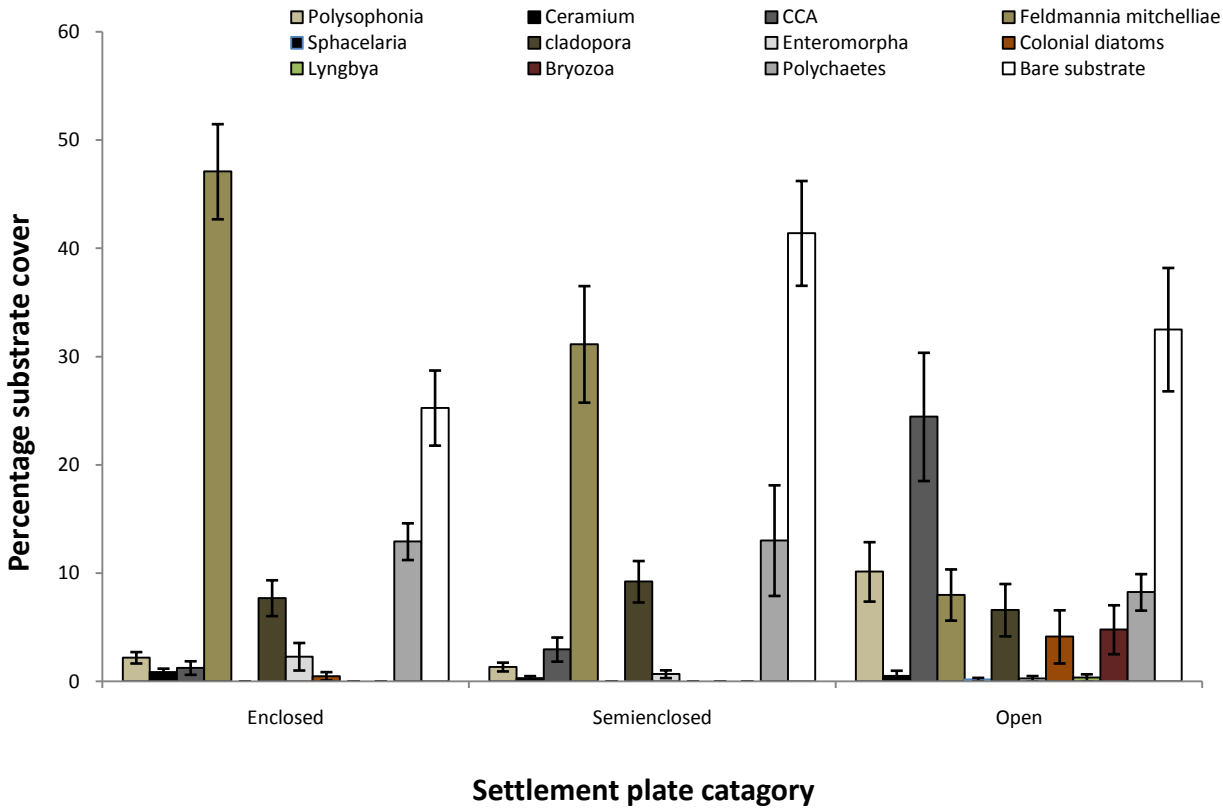

Figure 2.24 Species composition in enclosed, semi-enclosed and open settlement plates at Hall Bank reef (n = 41).

2.4 Discussion

Sea urchins are keystone species and are well known for their capability in adjusting their populations in response to nature of respective habitat that they live (Levitan 1989). The flexibility of their tests enables sea urchins to alter their test size proportionately in response food availability (Ebert 1980; Ebert et al. 2014; Levitan 1991). Test size differences of *C. tenuispinus* at the two study sites could be attributed to the varying level of food availability and differences in nutritional quality of food at the two sites. Minden Reef is a macroalgae-dominated patchy reef surrounded by seagrass meadows. Thus, *C. tenuispinus* populations inhabiting this reef have access to both macroalgae and seagrass as food sources. In contrast, Hall Bank reef was dominated by a biofilm consisting of filamentous microalgae, "turf algae" and crustose coralline algae, resembling the nature of the substrate of sea urchin barrens. Higher proportions of calcareous material in gut contents indicates the active grazing by *C. tenuispinus* at Hall Bank reef (see Chapter 5). A possible reason for active grazing by *C. tenuispinus* could be food scarcity at Hall Bank reef. In contrast to Minden Reef, substrate composition at Hall Bank reef was less variable year-round. Most urchin

barrens are also known to be stable unless there is a drastic change in the habitat (Hill et al. 2003). Further, turf and coralline substrate in barrens are known to inhibit kelp recruitment (Kennelly 1987).

Intense grazing of urchins impacts algae growth, reducing the competition for coral growth. This may be responsible for the higher coral cover at Hall Bank. Presence of macroalgae is known to be a typical feature of the reefs in this latitude. While Thomson and Frisch (2010) reported that Hall Bank reef has the highest mean coral cover (52.6± 4.65%) recorded at this latitude, this study recorded a mean coral cover of 12.61 ± 2.04%, which is one fourth of the value recorded by Thomson and Frisch (2010). The highest coral cover per transect recorded in this study (52.80%) was lower than the highest value recorded by Thomson and Frisch (2010) (72.5%). The large differences in coral cover since the study of (Thomson and Frisch 2010) can be attributed to temporal variations in substrate cover due to environment parameters such as water temperature. A marine heat wave occurred in the summer of 2010/2011, and caused reduced coral cover in many near-shore reefs across 12° of latitude in Western Australia (Moore et al. 2012a).

The north side of Hall Bank reef and the slopes were mainly dominated by turf algae, coralline algae and rubble. Rubble cover recorded from this study (21.69 ± 1.19%) was five times higher that of the study of Thomson and Frisch (2010) (4.1 ± 1.41%). Although coral cover was high on the south side of the reef, higher percentages of sponges contributed to substrate cover (9.48 ± 0.83%) as well, which was not recorded by Thomson and Frisch (2010). Crustose coralline cover (this study 9.8 ± 1.08 %) was also not included in the previous study. Instances of sea urchins excavating into the base of coral colonies were observed during this study (Figure 2.30E). Similar observations have been made at the Galapagos Islands with *Eucidaris galapengensis* (Glynn et al. 1979). Turf algae cover recorded in this study was slightly higher (44.27 ± 2.83%), than that recorded by Thomson and Frisch (2010) (40.5 ± 3.93%).

Another explanation for the low values for coral cover in this study is invasion of other invertebrates (sponges) or turf algae overgrowing corals (Figure 2.30D). Some turf algae species from family Dictyotaceae known to overgrow live corals despite their defensive mechanisms (Jompa and McCook 2003). Encrusting sponges act as bio-eroding agents in many reefs and they also are capable of overgrowing corals (Glynn and Manzello 2015). The comparatively low coral cover observed at Minden Reef is a typical feature of reefs at this latitude, which is mostly dominated by macroalgae

(Kendrick et al. 1999). The highest coral cover values (7.29 ± 4.16 %) recorded for Minden reef was close to those recorded by Richards et al. (2016)(6 ±2.1%).

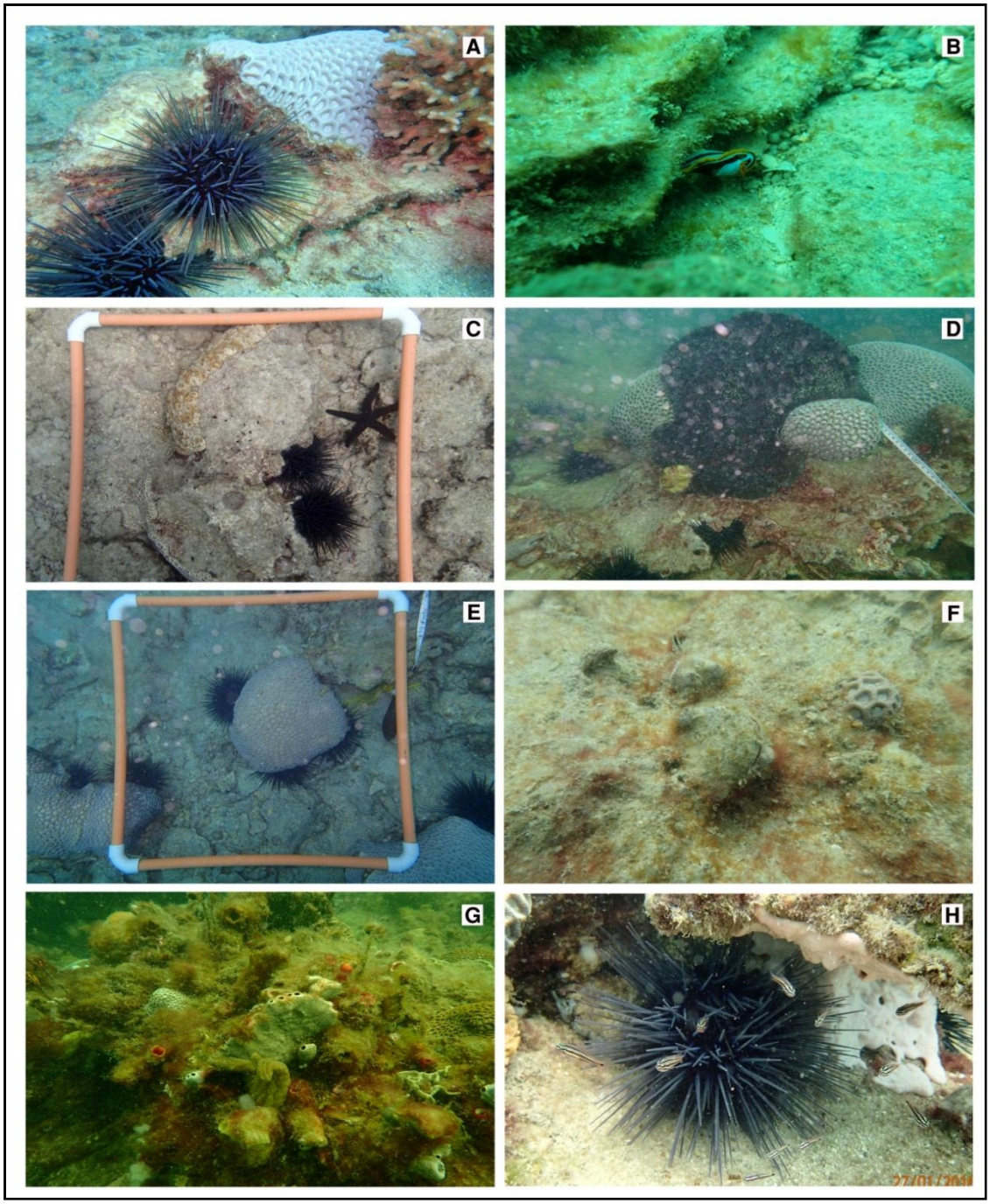

Figure 2.25 Photographs representing nature of substrate cover at Hall Bank reef (A/C/D: *C. tenuispinus* eroding in to coral colonies, B/F Turf algae, E: Sponge invading a coral colony) and Minden Reef (G: Turf and sponge cover in summer, H: *C .tenuipinus* eroding in to a sponge colony).

Out of 14 species of coral recorded by Thomson and Frisch (2010) only 12 species of coral were recorded in this study. *Porites lutea*, *Cyphastrea serailia*, and *Coscinaraea mcneili* were not recorded in this study. *Goniopora* sp. was not recorded by Thomson and Frisch (2010) at Hall Bank reef, but was recorded by (Antipas 2013)

and this survey. Low coral species richness could be due to the close proximity to the Australian mainland. Lord Howe Island which is 630 km away from mainland has 83 species of corals and the Abrolhos Islands, Western Australia, which is 60km away from the mainland has 184 species of corals (Veron and Marsh 1988). Hall Bank reef and Minden Reef are close to the Swan River mouth, and these reefs experience high turbidity outflows frequently, which is less favourable for coral growth.

Temporal variation in substrate composition at Minden Reef is mainly due to seasonal changes in macroalgae diversity and cover. Although many subtidal temperate reefs in Western Australian are dominated by kelp (*Ecklonia radiata*), Minden Reef was mainly dominated by foliose brown algae such as *Sargassum* spp. and *Dictyopteris* spp. Richards et al. (2016) reported that one site at Minden Reef was dominated by turf algae (57.5 ±12%)and *Ecklonia radiata* (24.3±14.5%), while another site was dominated by the seagrasses *Posidonia* sp. and *Halophila* sp. (49±12.8%). The higher percentage of *Sargassum* spp. in winter agrees with a study by Kendrick and Walker (1994).

Richards et al. (2016) recorded 188 species in surveys of Minden Reef including (12) coral species, octocorals (4), sponges (27), molluscs (48), echinoderms (16), crustaceans (34) and 46 spp. of fish. The high biodiversity observed at Minden Reef is due to high structural complexity providing more habitats for many species. In comparison Hall Bank reef is structurally less complex and less diverse. The majority of molluscs at Minden Reef are known to be filter feeders, and there are less herbivorous species at Minden Reef, which could be another reason for the high macroalgal cover. The main grazers at both sites were sea urchins and herbivorous gastropods. Sea urchin density recorded at Hall Bank reef was lower (2.94 ± 0.14 individuals m^{-2}) than the value (5 ± 0.8 individuals m^{-2}) recorded by Thomson and Frisch (2010) and higher than densities recorded in Rottnest Island (fished sites 0.10 - 1.93 m^{-2}; Sanctuary 0.87 - 1.83 m^{-2}) (Cook and Vanderklift 2011). Despite the high density of sea urchins and the presence of the herbivorous gastropod *Trochus* sp., turf algae cover is high at Hall Bank reef.

The existence of three sympatric urchin species (*C. tenuispinus*, *Phyllacanthus irregularis* and *Heliocidaris erythrogramma*) at Minden Reef clearly shows the high availability of resources in the habitat. Niche separation among coexisting urchins living within the same habitat has been observed elsewhere as well (Cobb and Lawrence 2005; Contreras and Castilla 1987; Vanderklift et al. 2006; Wangensteen et al. 2011). These three species are also known to co-exist in other subtidal reefs in Western Australia (Vanderklift et al. 2006). The dominance of C. *tenuispinus* at Hall Bank reef could be

mainly due to its exceptional ability to live in nutritionally poor habitats. Even with the presence of macroalgae this particular species tends to actively graze on attached algae (Vanderklift et al. 2006). A possible explanation for this behaviour could be due to the less palatable nature of macroalgae or difficulties in handling these algae species due to their physical structure (Stimson et al. 2007). Since Hall Bank reef is surrounded by seagrass beds (at 15m depth), drifting seagrass debris is another food source for species living in the reef system. However gut content analysis of *C. tenuispinus* revealed that seagrass is relatively unimportant as a food source (Chapter 4). Unlike *Heliocidaris erythrogramma* which depends on drifting algae, *C. tenuispinus* doesn't have elongated tube feet for trapping drift algae; therefore, even in the presence of drifting algae they tend to feed on substrate-attached forms. Sea urchins are well known for switching feeding habits in response to food availability (Harrold and Reed 1985). In addition to the omnivorous nature of this species, *C. tenuispinus* at Minden Reef is capable of altering its feeding habits (Chapter 4). *Centrostephanus rodgersii* is known to occupy a different realized niche in newly extended habitat in Tasmania (Perkins et al. 2015). In Tasmanian waters *C. rodgersii* barrens have become established in deeper waters (16 – 58 m) compared to New South Wales waters (7 – 27 m). This has changed the diversity and ecosystem functioning, negatively influencing other invertebrates and fisheries in Tasmania (Perkins et al. 2015).

Many invertebrates reproduce and increase in number when water temperature is high, leading to a high abundance of juveniles in summer and autumn (Edgar 1983). Diets of *C. tenuispinus* at Minden Reef reflect a shift in foraging behaviours (Chapter 4). Behavioural switches in feeding can lead to the destruction of kelp and the formation of "urchin barrens" in certain geographical regions (Filbee-Dexter and Scheibling 2014; Flukes et al. 2012; Harrold and Pearse 1987). Conditions leading to barrens in a particular location do not always drive the formation of barrens in other locations (Byrnes et al. 2013b). As Byrnes et al. (2013b) suggested, urchin density, level of kelp consumption, the variability of urchin consumptive potential and variability in kelp abundance are the main factors that need to considered to predict the urchins potential for phase shifts from kelp to barrens.

Many studies have suggested that sea urchin size is dependent on food availability, population density and level of predation. The most important finding of this study was differences in size and density of the *C. tenuispinus* population at the two study sites, Hall Bank and Minden Reef. Higher densities of comparatively small/intermediate sizes of *C. tenuispinus* at Hall Bank resemble the urchin populations

of food-scarce habitats such as barrens. In contrast, larger urchins at Minden Reef and low densities show the features of urchin populations in macroalgae / kelp-dominated reefs. Many sea urchin species are capable of adjusting skeletal test, body mass and gonads, proportionally reducing their energy costs (Levitan 1991). Food scarcity at Hall Bank reef could be the main reason for the small size of urchins. Sea urchins inhabiting food-constrained barrens tend to feed on microbial films and encrusting coralline algae, while when they inhabit kelp forests they mainly feed on macroalgae, leading to size differences with respect to habitat (Johnson and Mann 1982; Ling and Johnson 2009; Scheibling et al. 1999). An inverse relationship between mean body size and population density has been observed in *Diadema antillarum* (Levitan 1991; Levitan 1989). Similarly, Tuya et al. (2004) observed a relationship between body size and the population density of *D. antillarum* in the Canarian Archipelago, which revealed environments with a high density of individuals dominated by small-to-intermediate sized sea urchins, whereas low sea urchin density locations were dominated by large-sized individuals. A similar outcome was observed in this study. Having larger test diameters in macroalgae-dominated reefs and having smaller individuals in barren or low-food areas is characteristic of many sea urchin species. *Evechinus chloroticus* in New Zealand (Wing 2009), *Centrostephanus rodgersii* on the east coast of Australia, Tasmania and New Zealand (Byrne et al. 1998; Hill et al. 2003; Johnson et al. 2005; Ling and Johnson 2009), *Paracentrous lividus* in the Mediterranean, *Diadema antillarum*in the Caribbean (Bak et al. 1984; Lessios 1988b; Lessios et al. 2001; Levitan 1991) have been extensively studied for their capability to shift ecological states in the habitat. A classic example of a drastic change in urchin size was witnessed from Caribbean reefs; before the mass mortality (1983) 95% of *Diadema antillarum* population was in size range 20-40mm with a density of $15m^{-2}$. Three years after the mortality event (1987), 70% of its population ranged 60-80 mm at a density of 0.2 m^{-2} (Levitan et al. 1992).

Food scarcity in the habitat itself is reflected not only in small body sizes, but also the higher Aristotle's Lantern Indices. Many sea urchin species show differences in the sizes of jaws (demi-pyramids) of Aristotle's lantern with respect to test diameter, and these differences are dependent on food availability. The Aristotle's Lantern Index represents the extent of feeding efforts in depending on food availability (Agnetta et al. 2013; Ebert 1980; Ebert et al. 2014; Hagen 2008). When food is limited some echinoids are capable of enlarging their Aristotle's lantern compared to the body size (Coppard

and Campbell 2005a; Ebert 1980; Levitan 1991). This altered body state can be used to overcome food limitations in the environment (Levitan 1991).

Urchins at Minden Reef were cryptic in nature and tended to be under reefs or among spaces within the reef, whereas urchins at Hall Bank tended to be in more open areas; this could be associated with the level of habitat complexity and predator avoidance. Studies of the sea urchin *Strongylocentrotus purpuratus* suggest that microhabitat is a key factor determining growth and behaviour of bio-eroding sea urchins (Hernández and Russell 2010). As a result of high flexibility in urchins' tests, rapid changes in growth (i.e.test diameter; height ratios, ALI) within short periods of time have been documented. Thus, the shape of the urchin test confirms the microhabitat most recently occupied (Hernández and Russell 2010). Although there are few studies focused on mineralogy and the physical nature of the substrate, this plays a major role in urchin test shape as well (Hernández and Russell 2010). Lack of correlation was observed between substrate cover in urchin density in both sites (except with turf algae cover at Minden Reef) indicates that grazing is not always linearly related to urchin density. Hill et al. (2003) suggested that a threshold density of sea urchins is essential to prevent algae growth significantly.

The dominance of large individuals at Minden Reef and dominance of small/intermediate urchins at Hall Bank reef is likely directly related to food availability. The absence of smaller size classes at both sites (Hall Bank < 38 mm, Minden Reef < 83 mm) was notable. The scarcityof small individuals of *C. tenuispinus* was also observed by Vanderklift and Kendrick (2004) at Stragglers Rocks, Mewstone Rock and Carnac Island. A few reasons could explain the absence of small individuals, such as the cryptic behaviour of small urchins, low recruitment or lower settlement of juveniles. Microbial films are known to induce metamorphosis of many regular echinoids. High settlement of *Strongylocentrotus droebachiensis* and *Paracentrotus lividus* in response crustose coralline algae and bio-films are well known (Brundu et al. 2016; Pearce and Scheibling 1991). Settling larvae are known to respond to surface texture and contours as well. The Hall Bank substrate consisted of a bio-film of filamentous algae such *Polysiphonia* spp. and *Ceramium* spp., which is a cue for larval settlement. (Pearse 1970) suggested that large filter feeders such as bryozoans and larger *Spirorbis* sp. are capable of feeding on echinoplutius larvae and eggs with active filter feeding. Although filter feeding species such as polychaetes and bryozoans were present at both study sites, it is less likely they predate upon urchin larvae since their sizes and densities were low.

In certain areas larval settlement doesn't occur annually (Moore et al. 1963). The scarcity of small sized individuals has been recorded in red and purple urchin populations in southern California and conspicuous settlement is known to occur in 1-4 year intervals in these regions. In contrast, Tegner and Dayton (1981) observed recruitment of *Mesocentrotus franciscanus* in three consequent years and they also witnessed higher recruitment in deeper sites than shallow sites.

There have been no previous studies on the larval ecology of *C. tenuispinus*. Larval development of *C. rodgersii* is similar to other sea urchins within the family Diadematidae, although it has a long pelagic larval stage (110-120 days) compared to other diadematoids. A laboratory study revealed survival of only 6 individuals out of 2500 larvae, indicating low survival rates in laboratory conditions (Huggett et al. 2005). *C. rodgersii* in northern New Zealand are known to grow rapidly in the first year (23.8mm per year), then slow growth at 1.4 years and growth rate decreased when they reached an asymptotic size at 10-15 years (Pecorino et al. 2012). This initial lag in growth is believed to be related to dietary constraints. They are known to occupy habitats such as under boulders and crevice feeding on algal films and drift algae. A possible explanation for the scarcity of *C. tenuispinus* juveniles could be their cryptic behaviour, similar to *C. rodgersii*. The high dominance of intermediate urchins in *C. tenuispinus* population could be due to growth limitation influenced by low food availability; however, growth rates need to be measured for better understanding of differences in test sizes at the two study sites. The *C. tenuispinus* populations at Minden Reef and Hall Bank reef showed a unimodal size distribution, yet the population at Hall Bank had more small to intermediate urchins, while the population at Minden Reef had a higher proportion of larger individuals, indicating high food availability at Minden Reef. Although small individuals are scarce at Minden Reef, once the juvenile urchins progress past their cryptic phase they must have high growth due to the high availability of food. In contrast, they maintain a stationary mode in growth in response to low food availability at Hall Bank reef. The dominance of intermediate urchins has been recorded in many populations with low larvae supply, low recruitment or high predation rates (Sala and Zabala 1996). On the other hand higher proportions of small individuals reflect higher recruitment and lower predation on small sizes (Sala and Zabala 1996; Shears and Babcock 2002).

Populations subject to high predation are known to exhibit bimodal population size structure, when the intermediate urchins are vulnerable to predators and when there are spatial shelters against predation for the small sizes and shelter in size for the larger

ones (Tegner and Dayton 1981). Similar trends have been observed in other sea urchin species, where large sea urchins are found in protected areas (Shears and Babcock 2002; Tuya et al. 2004). Low complexity of reef habitat (e.g. *Pocillopora* sp.) also provides very little shelter for sea urchins from predators, compared to reefs that have a greater presence of massive species like *Porites lobate* (of higher complexity) (Palacios and Zapata 2014).

Although fish species like the blue lined leather jacket (*Meuschenia galii*), brown spotted wrasse (*Notolabrus parilus*, male western king wrasse *Coris auricularis* and western fox fish (*Bodianus frenchii*) are known to feed on other co-existing urchin species, only the western blue grouper (*Achoerodus gouldii*) is known to feed on adult *C. tenuispinus* successfully. Lobsters are known to predate on sea urchins in many regions (Tegner and Levin 1983), however sea urchins are not favoured by the Western rock lobster (*Panulirus cygnus*) in this region (Dumas et al. 2013). An absence of dominant predators could be favouring the observed size structure of *C. tenuispinus* at both study sites. Population size and structure of *C. tenuispinus* in these study sites therefore could be driven by food availability, rather than predator pressure. Impact of predation was not focused on this study. Although fish survey results showed a lack of major sea urchin predators in two study sites, it is essential to conduct detailed predation experiments with tethering urchins. Sea urchins are capable of changing habitat structure and composition within a short period of time (Abraham 2007; Filbee-Dexter and Scheibling 2014; Flukes et al. 2012; Ling et al. 2009b; Scheibling et al. 1999). The sea urchin exclusion experiment in this study suggests that *C. tenuispinus* is responsible for shifting species composition through grazing. As evidenced by this study, the dominance of a single or few species in the absence of herbivore pressure has been recorded in many geographical regions (Paine and Vadas 1969; Sammarco 1982; Wright et al. 2005). Habitats subjected to moderate levels of grazing are known to have higher species richness. On the other hand, intense urchin grazing is known to promote domination of crustose coralline algae which is a characteristic feature of urchin barrens.

Sea urchins, as keystone species in many marine benthic habitats, influence the structure and functioning of the ecosystem (Adam et al. 2015; Bulleri et al. 1999; Byrnes et al. 2013b; Harrold and Pearse 1987; Harrold and Reed 1985; Humphries et al. 2014). The absence of these species could lead to drastic changes in the habitat itself (Carpenter 1990; Scheibling 1986). This study confirms that *C. tenuispinus*, being a

principal grazer at Hall Bank reef, is critically important to the structure and functioning of the reef.

Chapter 3 - Reproductive Biology of *Centrostephanus tenuispinus*

3.1 Introduction

3.1.1 Sea urchin reproduction

Sea urchins are unique among other echinoderms not only due to their morphology but also due to their key role in many shallow marine ecosystems as extensive grazers (Andrew et al. 2003; Bak et al. 1984; Brady and Scheibling 2005; Byrnes et al. 2013b; Carpenter 1988; Hughes et al. 1987; Lessios et al. 1984b; Lessios et al. 2001). Sea urchin gonads "roe", being an economically important fisheries resource, 117,000 tonnes of sea urchins are harvested annually worldwide (Andrew et al. 2003; Keesing and Hall 1998; Scheibling and Mladenov 1987). Since, they are both ecologically and economically important, reproductive biology of regular echinoids has gained considerable attention and has been well-studied in many diverse habitats around the world for several decades (Arafa et al. 2012; Bronstein et al. 2016; Byrne et al. 1998; Drummond 1995; Himmelman 1978; Iliffe and Pearse 1982; Paine 1969).

Although sexual dimorphism is inconspicuous, regular echinoids are dioecious and basically broadcast spawners (Giese et al. 1987). Gametes are produced in the gonads and releasing gametes through spawning is a very common strategy among sea urchin species. The duration of gamete production and seasonality of gamete release/spawning varies considerably, not only among different species but also within the same species in different geographical regions (Bronstein et al. 2016; Iliffe and Pearse 1982; Lawrence 2013; Lessios 1981; Randall et al. 1964). These variations coincide with internal or external factors that influence gonad growth and gamete release or spawning. Proper understanding of urchin reproductive biology and spawning seasonality is key to explaining recruitment patterns of a particular species.

Many previous studies on reproductive biology are based on analysing percentage gonadosomatic index (GSI) and histology of gonads (Arafa et al. 2012; Gonor 1973; Grant and Tyler 1983). GSI depicts the percentage of gonad weight to the total weight of the organism. Generally, GSI increases with the development of gonads, and it is highest immediately prior to spawning (Arafa et al. 2012). Hence, changes in GSI have been used to predict the spawning seasonality of species in many studies (Arafa et al. 2012; Grant and Tyler 1983). Grant and Tyler (1983) suggested that GSI is not an indicator of spawning periodicity in some species and when a wide range of sizes is sampled, however. Further, they suggested combining histological analysis of gonads with the GSI is a more reliable method to predict gonad maturity stage and spawning

season. Methods such as gonad retrieval rate (regression between gonad weight against total weight) in combination with histological studies have also been used to predict spawning seasonality of sea urchins (Byrne et al. 1998).

GSI/gonad retrieval rate can vary depending on availability of food and its nutritional value (Meidel and Scheibling 1998; Meidel and Scheibling 1999; Tsuda et al. 2006). Higher GSI values have been recorded in habitats with abundant food/algae compared to areas of food/algae scarcity (Kelly 2001; Meidel and Scheibling 1998; Minor and Scheibling 1997; Sánchez-España et al. 2004). Sea urchins from kelp beds have been documented to have higher GSI compared to the same species from barrens (Gonor 1973; Meidel and Scheibling 1998). On the other hand, reproductive effort of *Diadema antillarum* is known to be density dependent (Levitan 1991; Levitan 1989). Thus, decreased gamete production due to low food availability can be compensated by increased fertilization success when urchin densities are high (Hernández et al. 2006).

3.1.2 Factors influencing sea urchin reproduction

The precise timing of spawning is critical to survival of any species. Variations in spawning patterns in sea urchins have been reported to depend on both spatial factors (geographical location) and temporal factors (seasonality) (Alsaffar and Lone 2000; Bronstein et al. 2016; Pearse 1969). Some species with broad latitudinal ranges have been observed to have extended breeding seasons (Bronstein et al. 2016; Giese and Pearse 1974; Hori et al. 1987; Lawrence 2013; Tuason and Gomez 1979), while species restricted to high latitudes reproduce during only a limited time of the year (Byrne et al. 1998). Some urchin species which have a broader geographical range are known to have mature individuals year round, but spawning of the majority of the population is known to be seasonal in high latitudes (Pearse and Phillips 1968).

Numerous factors have been shown to induce spawning of echinoderms: photoperiod or light intensity (Alsaffar and Lone 2000; Pearse et al. 1986; Shpigel et al. 2004), time of day, sea water temperature (Bronstein et al. 2016; Byrne 1990; Coppard and Campbell 2005b), phytoplankton/phytodetritus abundance (Bronstein et al. 2016), lunar cycle (Coppard and Campbell 2005b), tides/currents (Boolootian et al. 1959; Coppard and Campbell 2005b), salinity (Giese et al. 1964), inter-population and inter-individual communications (Giese and Pearse 1974; Lamare and Stewart 1998). Individual or combinations of multiple factors are capable of influencing spawning of particular sea urchin species in a particular geographical region. Further, depending on the environment and fluctuation of these factors, the frequency of spawning varies as well (Alsaffar and Lone 2000; Giese et al. 1987).

Evidence suggests that among other factors sea water temperature and photoperiod/day length play a major role in the initiation of gametogenesis and spawning of sea urchins in high latitudes (Alsaffar and Lone 2000; Byrne 1990; González-Irusta et al. 2010). Day length is an ideal factor to synchronize the reproduction since it is less variable to given latitude in the same period (Giese and Pearse 1974). Short days are known to induce gametogenesis, while long days are known to inhibit gametogenesis (Hernández et al. 2011). Gametogenesis of *Paracentrotus lividus* on the west coast of Ireland is initiated by both sea water temperature and photoperiod (Byrne 1990). Bronstein and Loya (2015) suggested that gametogenesis of *Echinometra* sp. in Gulf of Aqaba, Red Sea is induced by short day length and spawning may be driven by sea water temperature. In contrast, Drummond (1991) and Pearse and Cameron (1991) suggested that sea water temperatures do not play a central role in regulating reproductive cycles in Indo-Pacific sea urchins. Yet, it is evident that sea water temperature has a role in the reproductive cycles of *Diadema savignyi* in Fiji (Coppard and Campbell 2005b), *Diadema setosum* in Kuwait (Alsaffar and Lone 2000), *Diadema antillarum* in Bermuda (Iliffe and Pearse 1982), and *Echinometra mathaei* in Kuwait and Egypt (Pearse 1969). Laboratory experiments have also demonstrated the impact of temperature on GSI (James and Heath 2008). Although sea water temperature may not impact the reproductive cycles of tropical urchins, seasonal changes in sea water temperature can impact reproduction in high latitude urchins. Further, it is evident that temperature plays a major role in larval development. Investigating the potential influence of variable growth and development of planktonic larvae of *Strongylocentrotus droebachiens is* in response to temperature and food abundance, Hart and Scheibling (1988) revealed that growth was rudimentary within the range 3°C to 9°C, and larvae grew most rapidly at 14°C.

The pattern of reproduction periodicity varies between species and can be synchronized within a population in a specific geographical location (Lessios 1981). Populations of *Diadema mexicanum* and *Echinometra vanbruuti* in the Bay of Panama display well-defined synchronous reproductive cycles; however reproductive periodicity was less defined on the less seasonal Caribbean coast (Lessios 1981). *Tripneustes gratilla* in the Indo-West Pacific exhibits an annual reproductive cycle with variable intensities (Vaitilingon et al. 2005). Its reproductive cycle is mediated by seawater temperature, day length and feeding activity. Many seasonal spawners have highly synchronized reproductive cycles within the two sexes and across populations (Brewin et al. 2000; Byrne et al. 1998). *Centrostephanus rodgersii* on the east coast of Australia

(latitude range of 7°) has a highly synchronized reproductive cycle near its northern limits and a less synchronized cycle near its southern limits (Byrne et al. 1998). This species spawns in response to the winter solstice with low sea water temperature and short day length (Byrne et al. 1998; King et al. 1994). On the other hand, *Evechinus chloroticus* in New Zealand and *Heliocidaris erythrogramma* in Australian waters initiate spawning in spring/summer (Brewin et al. 2000; McShane et al. 1996; Walker 1982).

3.1.3 Significance of *Centrostephanus tenuispinus*

Many high latitude sea urchin species (*Paracentrotus lividus* in the Mediterranean, *Strongylocentrotus* spp. in North America and Japan, *Psammecanus miliaris* in Europe, *Evechinus chloroticus* in New Zealand) have been extensively studied due to their economic value (Andrew et al. 2003; Andrew and MacDiarmid 1999; Byrne 1990; Shpigel et al. 2005). Among southern temperate species, *Evechinus chloroticus, Heliocidaris erythrogramma* and *Centrostephanus rodgersii* have been well-studied during the last few decades, not only for their potential economic value but also for their ecological role (Andrew 1988; Brewin et al. 2000; Kailola 1993; King et al. 1994). *Centrostephanus rodgersii* has attracted considerable attention due to its recent range expansion, impact on ecology of newly established habitats and its potential as a fishery resource (Byrne et al. 1998; King et al. 1994; Ling 2008; Ling et al. 2008; Ling et al. 2009b; Pecorino et al. 2013a; Pecorino et al. 2013b).

Among the two *Centrostephanus* species in Australia, the ecology and biology of *Centrostephanus tenuispinus* on the west coast of Australia is poorly known. No previous studies have been conducted on reproductive periodicity of *C. tenuispinus*. Studies so far on this species have been restricted to a feeding behaviour study (Vanderklift and Kendrick 2005; Vanderklift et al. 2006) and a few population studies (Cook and Vanderklift 2011; Thomson and Frisch 2010). Being omnivorous, *C. tenuispinus* is capable of inhabiting a wide range of habitats (Vanderklift et al. 2006). This species is closely related and shows similarities in morphology to its congener *C. rodgersii* in east coast of Australia (Fell 1975). Thomson and Frisch (2010) suggested that *C. tenuispinus* is the main cause for the absence of macroalgae at Hall Bank reef, Western Australia, which is very unusual for a temperate reef. The correlation between high abundances of *C. tenuispinus* and low macroalgae at Hall Bank suggests the potential for the urchin to form and maintain barrens although it is evident that *C. tenuispinus* has an immense impact on its habitat; a lack of knowledge on its reproductive cycle hampers understanding of recruitment and behaviour of this

particular urchin. The main focus of this chapter is to understand the reproductive cycle, reproductive seasonality and factors impacting spawning of *C. tenuispinus* at Hall Bank reef, Western Australia. One of the interesting questions is whether there is a difference in reproductive output of *C. tenuispinus* depending on food availability of the habitat. Thus, this study compared reproductive output of *C. tenuispinus* in food scarce Hall bank reef with the macroalgae dominated Minden Reef. Monthly percentage gonadosomatic index combined with histological analysis was used for analysis of gametogenesis and reproductive seasonality in two study sites.

3.2 Methodology

3.2.1 Study site:

This study was carried out from October 2014 to February 2016 at Hall Bank reef (32°2.002´S and 115°42.957´). Comparison of reproductive output between Hall Bank reef (coral dominated reef) and Minden Reef (32º 04.320′S and 115º 43.782′E) (a nearby macroalgae dominated reef) was carried out in winter 2016 and summer 2017 (see Chapter 2 for site descriptions).

3.2.2 Sampling Procedure

Monthly sampling was carried out from October 2014 to February 2016. At each sampling session, ~26 sea urchins were collected through scuba diving. The urchins were transported to the laboratory on ice. Water quality parameters (temperature, pH, DO, salinity and conductivity) were measured during each collection using a portable YSI water quality meter (Model: 556 MPS). Water samples were collected and sent for analysis at the Marine and Freshwater Research Laboratory (MAFRL), Murdoch University for chlorophyll a, nitrate, nitrite and orthophosphate. Sea water temperature was obtained using two in situ temperature loggers (HOBO-UA-002-64) at Hall Bank reef and Minden Reef (data logger at Hall Bank deployed by CSIRO). Day length data were obtained from Geosciences Australia, Australian government.

Sea urchins were transported to the laboratory and dissected within the same day. Sea urchin samples were blotted dry and weighed with an electronic balance (± 0.001 g); test diameter and height were measured to two decimal places using Vernier callipers. Samples were dissected carefully, and Aristotle's lantern weight and height, gonad weight and gut weight were measured. One gonad from each sample was preserved in Bouin's fluid (formaldehyde: picric acid: glacial acetic acid 75 mL: 25 mL: 5 mL) for 24 hours and transferred into 70 % ethanol for histological analysis. Gonads were embedded in paraffin and sectioned (7 μm). Gonad sections were stained using the

haematoxylin-eosin aqueous method (Bancroft and Gamble 2008). Gonad sections were used to determine the gender and stage of maturity of the urchins. Gonad stages were classified into six maturity stages (Recovery, Developing, Premature, Mature, Partially spent, and Spent) following King et al. (1994).

3.2.3 Calculations and data analysis

3.2.3.1 Gonadosomatic index (GSI)

Gonad weight was used for calculation of Gonadosomatic Index (GSI). This indicates the size of gonads with respect to the body size as a percentage.

$$GSI = \frac{Gonad\ weight}{Total\ body\ wet\ weight}\ X\ 100$$

Maturity of each individual was estimated based on gonadal maturity stage. The sex ratio, as the proportion of females to the total, was estimated with respect to size and time.

3.2.3.2 Repletion Index (RI)

Repletion index, which is an indicator of gut fullness, was calculated according to the following equation for each individual;

$$RI = \frac{Gut\ wet\ weight}{Total\ body\ wet\ weight} X100$$

3.2.3.3 Comparison of reproductive output between Hall Bank reef and Minden Reef

Ten samples of sea urchins were collected from Hall Bank reef and Minden Reef in winter 2016 and summer 2017. Samples were dissected, and measurements were made as above, to calculate GSI. Histological analysis was carried out for each sample as well.

3.2.3.4 Statistical Analysis

Data were tested for normality (Levene's test). Differences in GSI between sexes and months were analysed using two-way ANOVA followed by post hoc tests (Tukey's HSD). Correlations between sea water temperature, day length and GSI, and between RI and GSI, were tested using Pearson's correlation coefficient. All statistical analyses were carried out using SPSS 24.

3.3 Results

3.3.1 Seasonal changes in Gonado-Somatic Index (GSI)

Reproduction of *Centrostephanus tenuispinus* is seasonal, and gametogenesis in males and females was synchronized in the *C. tenuispinus* population at Hall Bank reef. Mean GSI ranged from 2.76 ± 0.11 % to 6.89 ± 0.58 % (mean ± SE) and 2.95 ± 0.31 % to 6.93 ± 0.57 % (mean ± SE) for males and females respectively. Individual GSI recorded ranged from 1.37 % to 10.73 % for males and 0.32 % to 13.27 %. The highest mean GSI was recorded in July 2015 for both sexes (6.93 ± 0.57 (mean ± SE) for females and 6.89 ± 0.58 (mean ± SE) for males) (Figure 3.1). A clear increase of mean percent GSI from March to July was evident in both genders (females 5.30 ± 0.56 - 6.93 ± 0.57 %, males 4.47 ± 0.54- 6.89 ± 0.58 %).

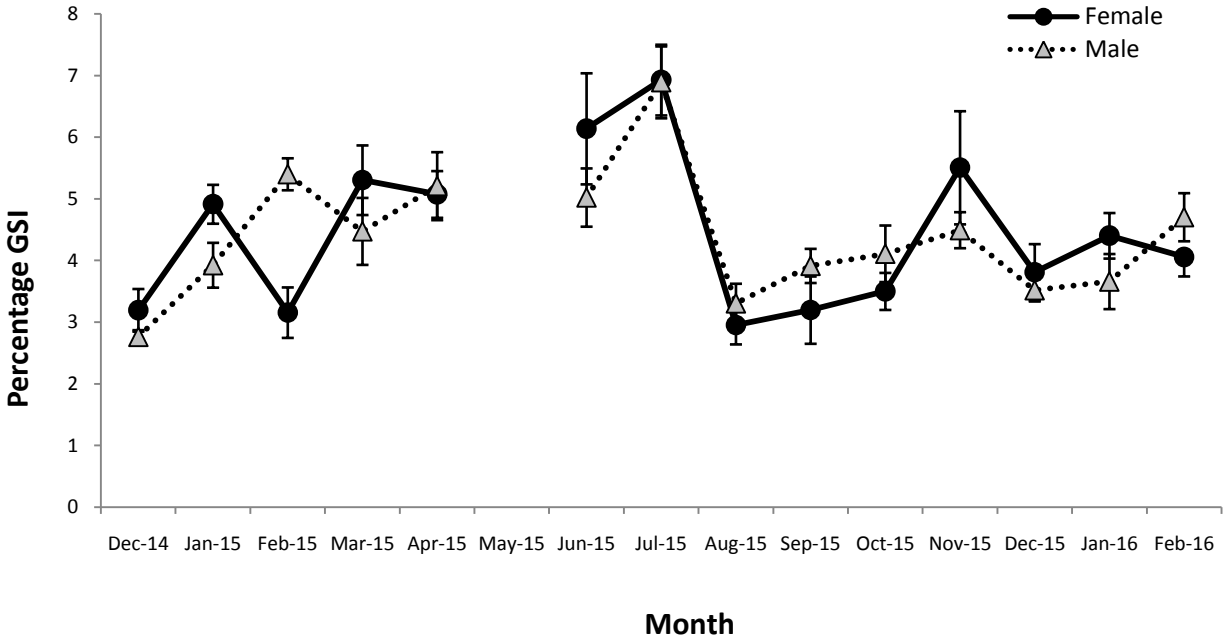

Figure 3.1 Monthly percentage Gonado Somatic Index (mean ± SE) of females (black line) and males (dotted line) of *C. tenuispinus* in Hall Bank Reef (Males; n = 208, Females; n = 182).

Two-way ANOVA revealed a significant interaction between gender and month ($F = 1.866$, $p = 0.028$) (Table 3.1), such that males had higher GSI values in February 2015 (5.40 ± 0.25 %), and females had higher GSI values in January 2015 (4.91 ± 0.31 %), March 2015 (5.30 ± 0.56 %), June 2015 (6.14 ± 0.90 %) and November 2015 (5.50 ± 0.91 %). Further, GSI of males in August, September and October were higher than female GSI. GSI in July was similar in both genders. Although there was a significant main effect of monthly percent GSI ($F_{(14,390)} = 9.812$, $p < 0.001$), no significant differences were observed between overall monthly male and female GSI ($F_{(1,390)} = 0.109$, $p = 0.741$).

Tukey's HSD test revealed that mean GSI for pooled data was significantly higher in July than the rest of the year (6.90 ± 0.02 %) (mean ± SE). Percent mean GSI in August 2015 was significantly lower compared to the first 7 months of the year and November (3.13 ± 0.18 %). Mean GSI in March, April and June were similar when gametogenesis was proceeding. Further, GSI in October 2014, September 2015 and October 2015 and December 2015 were also similar to the other months except March 2015 to July 2015 and November 2015. Mean GSI in November 2015 (5.00 ± 0.51 %) was significantly higher than the other months except January to July (period of gametogenesis). No significant differences were observed in GSI between years of 2014 and 2015 in the months of October ($p = 0.631$) and December ($p = 0.147$). Similarly, no significant differences were recorded between the year of 2015 and 2016 in January ($p = 0.400$) and February ($p = 0.824$).

Table 3.1 Source of variance table for the two-way ANOVA of mean percent GSI of gender and month as factors ($n = 390$), ($\alpha = 0.05$). * denotes significant.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	457.833[a]	29	15.787	5.768	< 0.001
Intercept	7232.658	1	7232.658	2642.571	< 0.001
Month	375.985	14	26.856	9.812	< 0.001*
Gender	0.299	1	0.299	0.109	0.741
Month*Gender	71.519	14	5.108	1.866	0.029*
Error	985.312	360	2.737		
Total	8919.661	390			
Corrected Total	1443.145	389			

3.3.2 Repletion Index

The highest repletion indices (RI) for females were in February 2015 (18.11 ± 0.60 %) and September 2015 (18.29 ± 0.10%). Similarly, the highest RI for males was in February 2015 (16.59 ± 0.62 %) and September 2015 (16.10 ± 0.61 %). The lowest RI was recorded in January 2016 for females (13.48 ± 1.15 %) and in January 2015 for males (12.16 ± 0.85 %) (Figure 3.2). Significant differences were observed between female and male RI (two-way ANOVA, $F = 11.151$, $p < 0.001$). Female RI was higher than male RI in most months. Further, monthly differences in % RI was ($F = 7.165$, $p < 0.001$) (Table 3.2).

Table 3.2 Source of variance table for the two-way ANOVA of mean percent RI of gender and month as factors (n = 390), (α = 0.05). * denotes significant.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	888.313	29	30.631	4.336	<0.001
Intercept	86466.461	1	86466.461	12239.232	<0.001
Month	708.672	14	50.619	7.165	<0.001*
Gender	78.780	1	78.780	11.151	0.001*
Month*Gender	107.056	14	7.647	1.082	0.372
Error	2543.291	360	7.065		
Total	91138.094	390			
Corrected Total	3431.604	389			

RI was higher for February 2015 and September 2015 compared to October 2014/15, July 2015, January 2015/16, and February 2016 (Tukey's HSD).

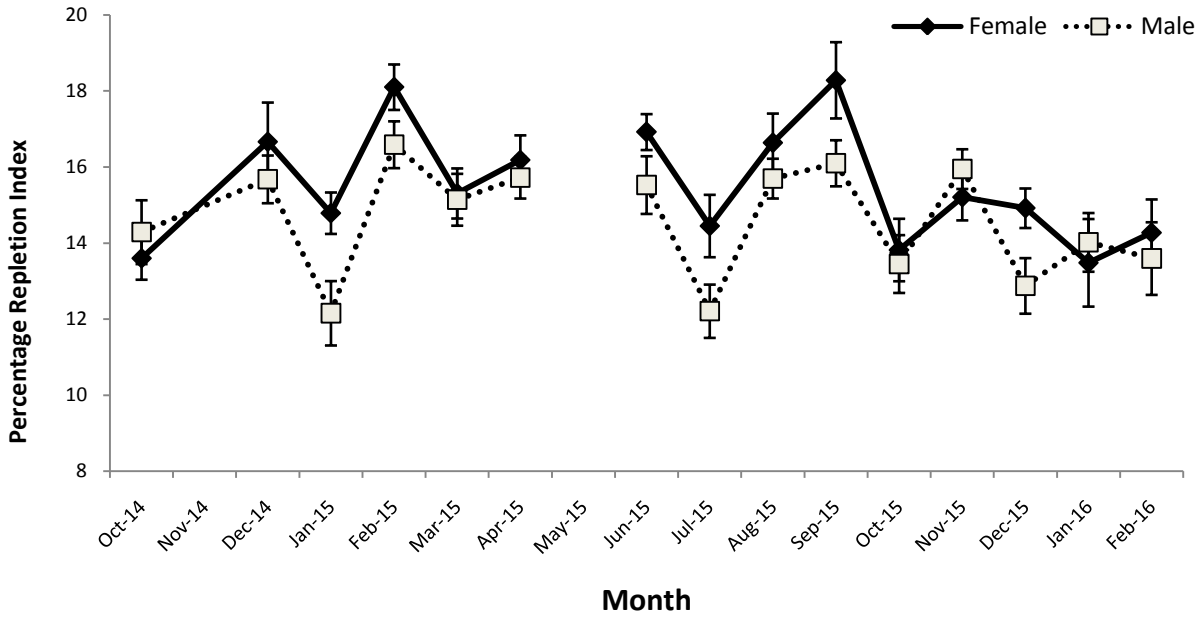

Figure 3.2 Monthly percentage RI (Mean ± SE) of C. tenuispinus (n = 390) from October 2014 – February 2016 Male RI and female RI represented by broken line and solid line respectively.

Pearson correlation revealed that there was no significunt correlation between % GSI and % RI for pooled data for genders (Pearson correlation = 0.008, p = 0.880)

3.3.3 Influence of sea water temperature and photoperiod on GSI

There was no correlation between male and female % GSI and sea water temperature (Males: Pearson correlation (PC) = -0.152, p = 0.122, Females PC = -0.137, p = 0.640). Male GSI is moderately correlated with day length (Pearson corelation -0.566, p = 0.035) (Figure 3.3).

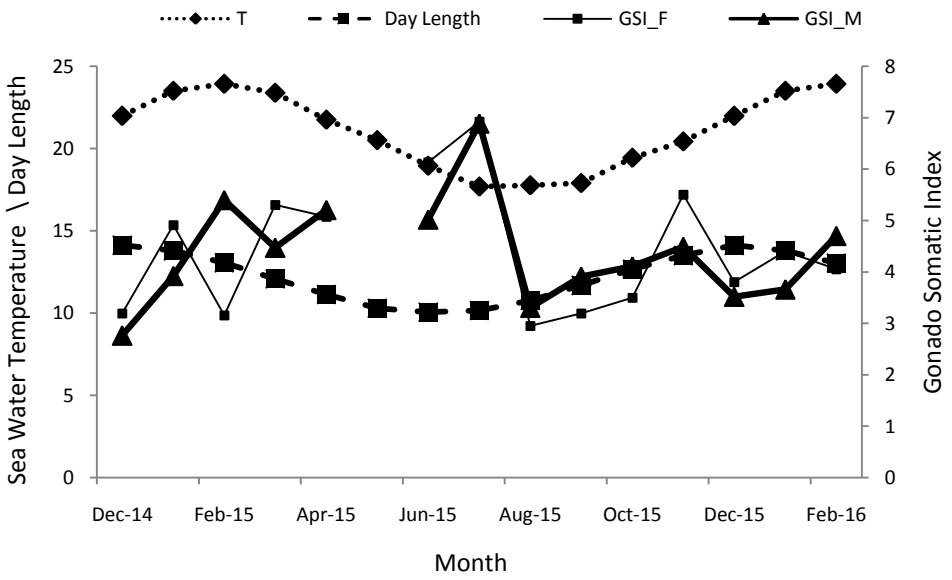

Figure:3.3 Monthly variation of sea water temperature (°C), day length (hrs) and % GSI of *C. tenuispinus* at Hall Bank reef.

3.3.4 Gametogenesis and gonad histology

Gonads of both genders were categorized into six stages: Recovery, Developing, Premature, Mature, Partially spent, and Spent, following King et al. (1994).

3.3.4.1 Oogenesis

Stage I: Recovery stage

The recovery stage can be further categorized into three stages. At the initial stage, all relict ova were subjected to re-absorption by nutritive phagocytes. Secondly, the lumen started to fill with densely eosinophilic nutritive phagocytes. Scattered brown pigments (lipofuscin) were present throughout the gonad tissue (Figure 3.5). Finally, occasional basophilic oocytes started to align with the tube wall. Initiation of primary oocytes was observed in December 2014 samples. While relict ova were being reabsorbed by nutritive phagocytes, at the periphery of the tubule occasional primary oocytes were appearing. Recovery phase was first observed in October 2015, two months after initial spawning, although no recovery stages were observed in October 2014 samples. All samples from December 2014 were in the recovery phase (100%), yet only 90 % of samples from December 2016 were in the recovery phase (Figure 3.4). All the samples from January and February of 2015 and 2016 contained gonads in the recovery phase.

Stage II: Developing stage

Developing basophilic oocytes lined most of the gonad wall forming a ring (Figure 3.5B). This process was accelerated in March. By April all individuals were in

the process of gametogenesis. Vitellogenesis progressed from April. These oocytes progressed towards the lumen utilizing the nutrients stored in nutritive phagocytes. Initially, these extended to an oval shape and tended to separate from the gonad wall and migrate towards the gonad lumen (Figure 3.5C). These ova consisted of a nucleus surrounded by nutrient stores. As they concentrated in the lumen, they tended to mature. All samples in April and June were in the developing phase, and 27% of the July population was composed of ova in the developing phase (Figure 3.4).

Stage III: Premature stage

At this stage, the majority of ova were concentrated in the gonad lumen (Figure 3.5D), although new oocytes were still developing along the tubule wall. With time, mature ova tended to occupy most of the lumen. Forty-six percent of the population was in the premature stage in July; July was the only month in which the premature stage was observed (Figure 3.4).

Stage IV: Mature stage

In this stage, the gonad lumen was full of mature ova, with the ova tightly packed and a very thin layer of nutritive phagocytes (Figure 3.5E). Mature ova first appeared in July (27.2 %) and were present in sampled populations from July to November (August – 20 %, September – 45.4 %, October – 30.7 %, November – 15.4 %) (Figure 3.4).

Stage V: Partially spent stage

Only 25-30 % of the gonad was spawned (Figure 3.7F). This stage was observed in October (23%) and November (15.4%) (Figure 3.4).

Stage VI: Spent stage

Gonads with empty lumens were considered as spent (Figure 3.5G). Occasional unshed ova were recorded at this stage, and nutritive tissue was absent. Eighty percent of samples were in the spent stage in August, and 54.5 % in September 2015, 38.5 % in October 2015, 38.5 % in November and 10% in December 2015 (Figure 3.4). No spent stage gonads were observed in the December 2014 samples, yet they were observed in December 2015. All the gonads entered recovery stage after November.

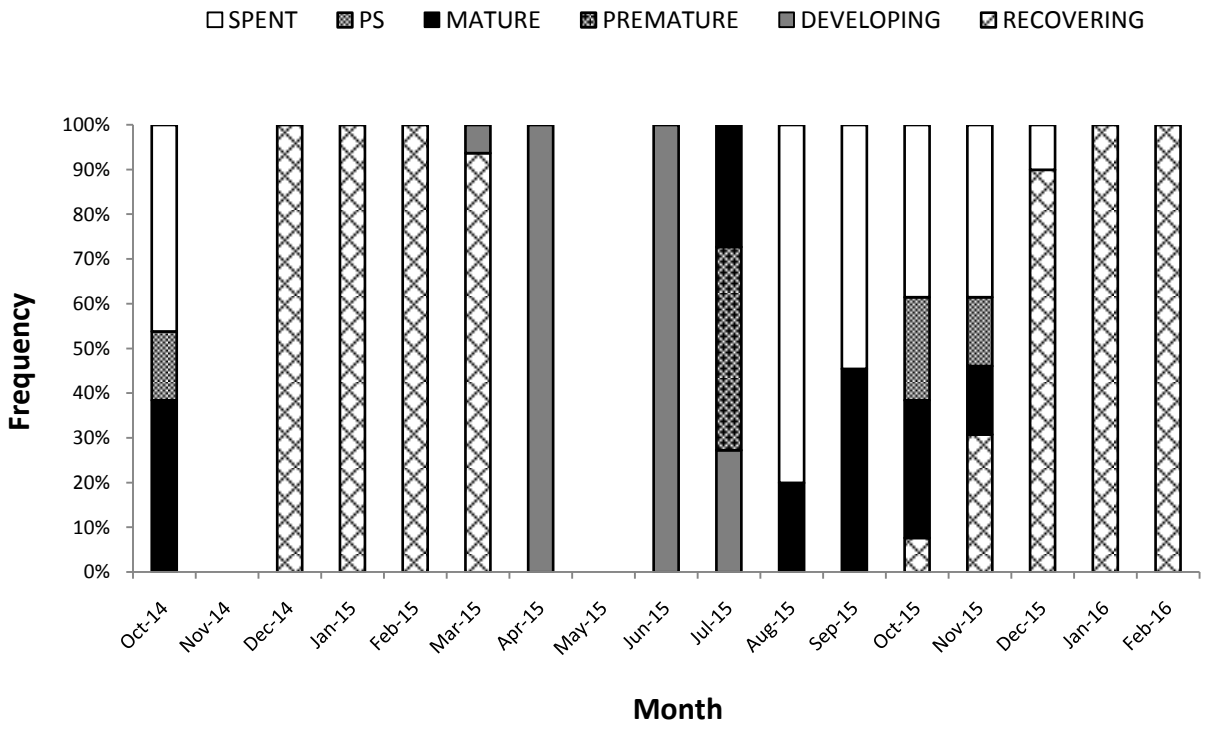

Figure 3.4 Temporal variation (October 2014 – February 2016) of percentage gametogenic stages in female *C. tenuispinus* (n = 182).

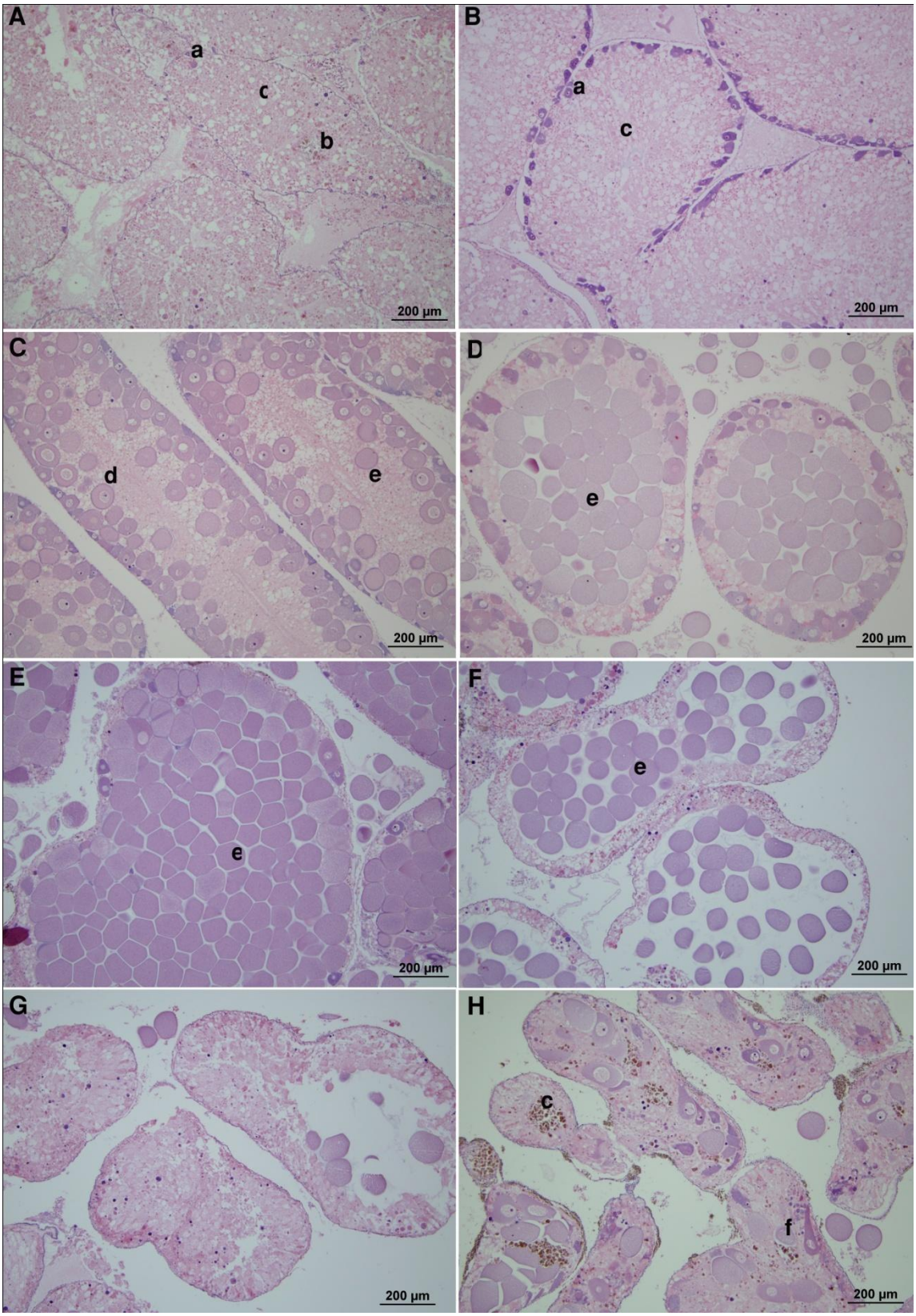

Figure 3.5 Histology of ovary of *C. tenuispinus* (A - Late Recovery stage; B – Initiation of oogenesis; C – Developing stage; D – Premature stage; E – Mature stage; F – Partially spent stage; G – Spent stage; H–Initial Recovery stage; a - Oocytes; b – Nutritive phagocytes; c - Lipofuscins; d – Developing ova; e – Mature ova; f – Relict ova).

3.3.4.2 Spermatogenesis

Stage I: Recovery stage

The lumen started to fill with densely eosinophilic nutritive phagocytes. Unshed sperms were re-absorbed. Brown pigments (lipofuscins) were scattered in the tubule lumen. Basophilic spermatozoa were aligning with the tubule wall (Figure 3.7A). Recovery stage in males was first observed in December (62.5%). Majority of the sampled population was in recovery stage from December to April (January – 66.7%, February and March – 100 %, April – 79.9 %). Last traces of recovery stages were observed in June (7.1 %) (Figure 3.6).

Stage II: Developing stage

Developing strands of sperms (basophilic) were observed (Figure 3.7B). These strands were arising along the tubular wall and tended to extend towards the lumen (perpendicular to the tubule wall). In April, 23 % of the sampled population was in developing stage. The majority of the population was in developing stage by June (92.8 %); 6.75 % of the July sample was also in late developing stage (Figure 3.6).

Stage III: Premature stage

At this stage, sperms had accumulated in the tubule lumen and were in the process of maturing (Figure 3.7D), but sperm strands continued to gather in the lumen. 46.7 % of the population was in premature stage in July and 12.5 % in August (Figure 3.6).

Stage IV: Mature stage

The gonad lumen was completely filled with basophilic mature sperms. Clusters of basophilic sperms in sections were visible to the naked eye. By this stage, the gonad wall and nutritive phagocytes layer were very thin (Figure 3.7E). The presence of mature sperms in July (46.7 %) indicates readiness for spawning. Mature sperms were present from July to December 2015 (August – 43.8 %, September – 46.8 %, October – 61.6 %, November – 38.5 %, December – 6.3 %) over six months. The highest percentage of mature stage testes was observed in October (Figure 3.6). Note that there was a complete absence of mature sperms in the December 2014 sample.

Stage V: Partially spent stage

Gonads with over 70 % of sperms remaining were considered as partially spawned. This stage was observed in August (25 %), September (13.3 %), October (15.4 %), November (23.8 %) and December (25 %) (Figure 3.6).

Stage VI: Spent stage

Spent testes were first observed in August (18.5%) (Figure 3.6). The highest percentage of spent testes from the sampled population was recorded in September (40 %), and this stage was present until January (for six months).

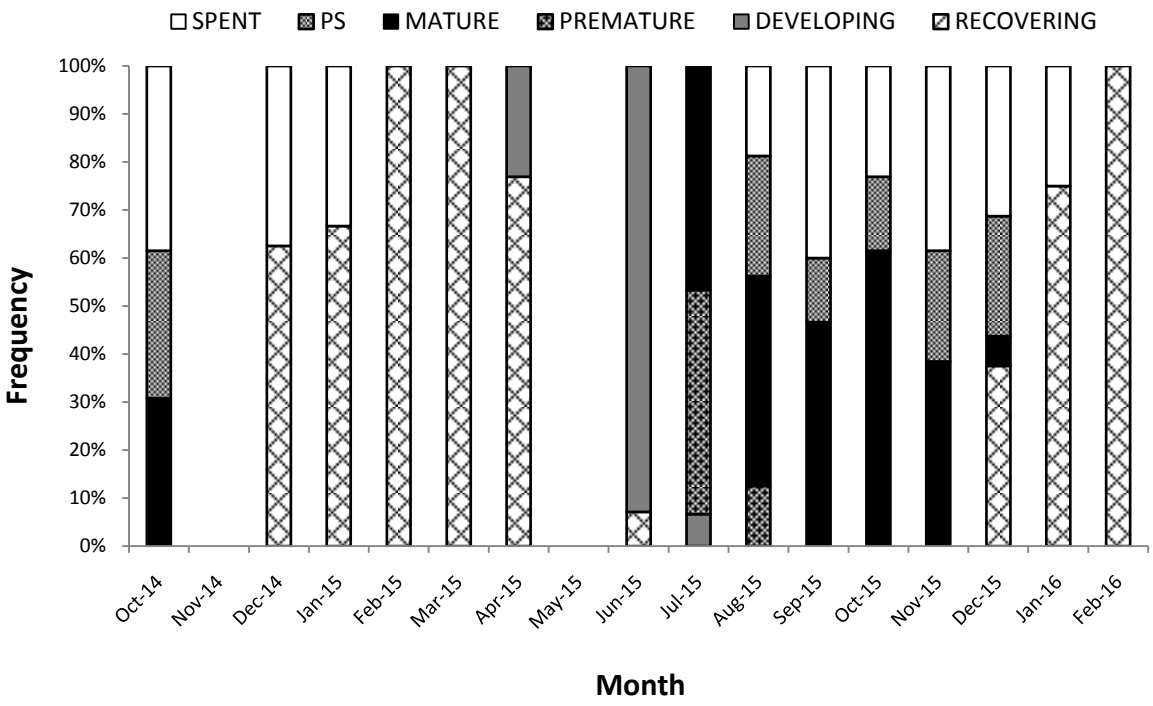

Figure 3.6 Temporal variation (October 2014 – February 2016) of percentage gametogenic stages in male *C. tenuispinus* (n = 208).

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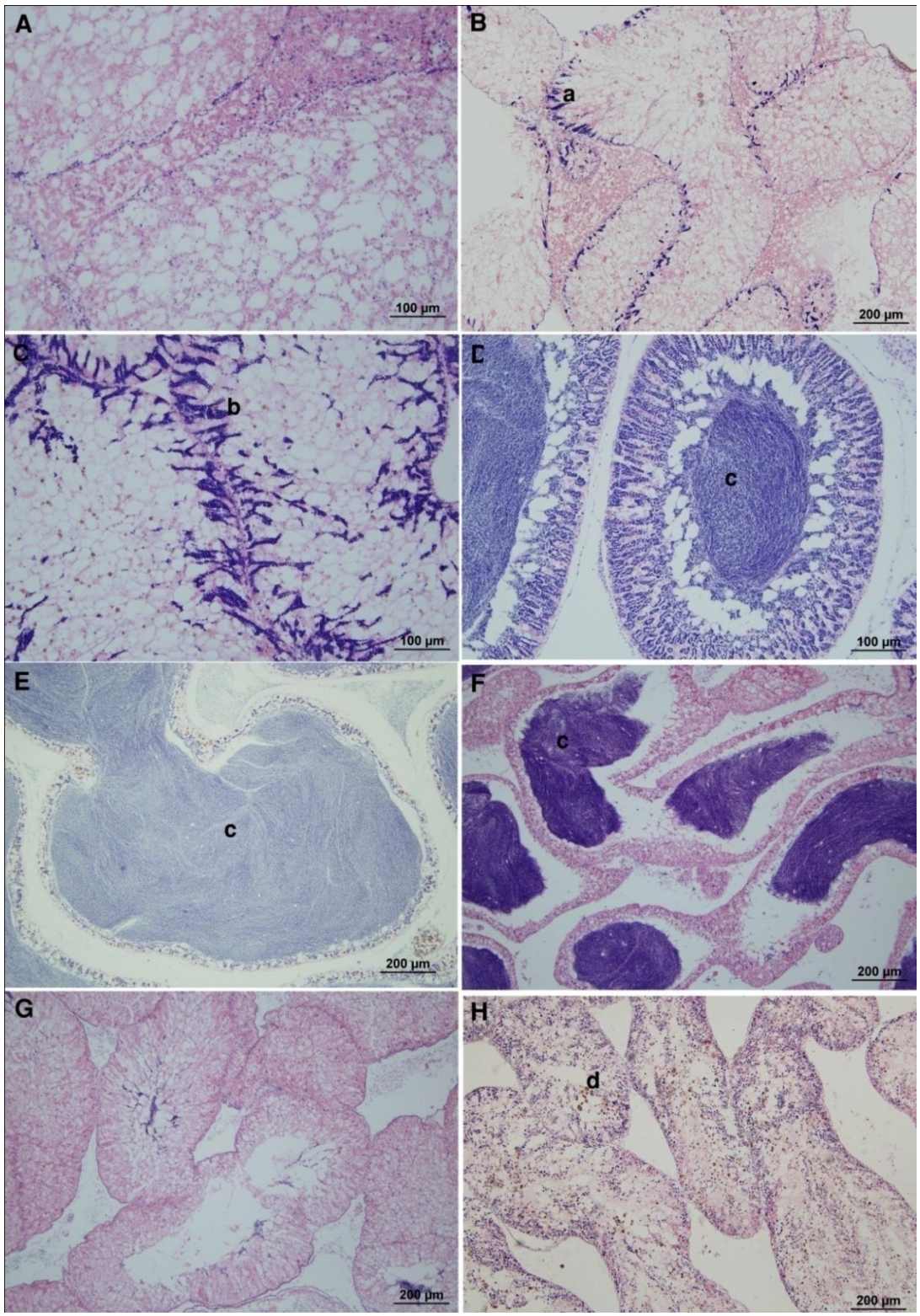

Figure 3.7 Histology of *C. tenuispinus* testes (A – Late Recovery stage; B – Initiation of spermatogenesis; C – Developing stage; D – Premature stage; E – Mature stage; F – Partially spent stage; G – Spent stage; H – Initial Recovery stage; a – Initiating sperm strands; b – Developing sperm strands; c – Mature sperms; d – Lipofuscins).

3.3.5 Sex ratios

Variations in sex ratios (female/male) were observed throughout the year, but remained close to 1:1 (χ^2, p = 0.19).

3.3.6 Comparison between sea urchins from Hall Bank and Minden Reef

The sea urchins collected from Minden Reef were larger than urchins from Hall Bank reef (Table 3.3) ($F_{(1,40)}$ = 252.383, p < 0.001). No seasonal difference was observed within each site with respect to test size ($F_{(3,40)}$ = 1.283, p = 0.265).

Percent GSI of sea urchins from Hall Bank reef ranged from 2.13 to 4.16% and 1.88 to 5.46 % in winter and summer respectively. Urchins from Minden Reef had relatively large gonads (two times and three times of % GSI of Hall Bank in winter and summer respectively). Two-way ANOVA revealed a significant interaction effect between site and seasons ($F_{(1,40)}$ = 17.038, p < 0.001) (Table 3.4), with higher % GSI values in Minden Reef compared to Hall Bank. Further, significant main effects were present with respect to both site and season. Sea urchins from Minden Reef had higher % GSI compared to urchins from Hall Bank Reef ($F_{(1,40)}$ = 91.50, p < 0.001) (Table 3.4). Similarly, winter had higher % GSI over summer (Table 3.3) ($F_{(1,40)}$ = 22.391, p < 0.001).

In winter, male urchins from Hall Bank reef were partially spent (66.66 %) and spent (33.33 %), and all females were in spent stage (100 %). All individuals sampled in summer from Hall Bank reef were in the recovery stage. This indicates similar patterns in *C. tenuispinus* reproductive cycle in 2014 and 2015 (section 3.3.4).

Samples from Minden Reef consisted of mature, partially spent, spent and recovery stages in winter and samples from summer comprised of individuals with recovery stage, indicating synchronized reproductive patterns with population in Hall Bank reef.

Repletion index in Minden Reef was 17.84-27.72 % and 11.43-19.06 % in winter and summer respectively. Hall Bank reef RI was 13.85-17.83 % in winter and 13.76-18.26 % in summer. Mean RI was higher in Minden Reef urchins than that of urchins from Hall Bank reef in winter and summer (Table 3.3/3.4/3.5) ($F_{(1,40)}$ = 16.033, p < 0.001).

Table 3.3 Comparison of GSI of *C. tenuispinus* (mean ±SE) and RI (mean ± SE) between Hall Bank reef and Minden Reef in winter and summer (n = 40).

	Winter		Summer	
	Hall Bank reef	Minden Reef	Hall Bank reef	Minden Reef
Mean GSI (%)	3.32±0.32	6.28±0.7	3.65±0.30	11.08±0.7
Mean RI (%)	15.14±0.57	21.63±0.99	15.65±0.67	14.91±0.81
Mean Test diameter (mm)	68.5±1.3	98.9±2.36	68.3±1.6	95.1±1.8

Table 3.4 Source of variance table for the two-way ANOVA of mean percent GSI of Site (Hall Bank reef and Minden Reef) and season (winter and Summer) as factors (n = 40), (α = 0.05). * denoted significant.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	385.445[a]	3	128.482	43.793	<0.001
Intercept	1479.363	1	1479.363	504.241	<0.001
Site	269.767	1	269.767	91.950	<0.001*
Season	65.691	1	65.691	22.391	<0.001*
Site*Season	49.987	1	49.987	17.038	<0.001*
Error	105.618	36	2.934		
Total	1970.427	40			
Corrected Total	491.063	39			

Table 3.5 Source of variance table for the two-way ANOVA of mean percent RI of Site (Hall Bank reef and Minden Reef) and season (winter and Summer) as factors (n = 40), (α = 0.05). * denoted significant.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	310.820	3	103.607	17.135	<0.001
Intercept	11328.591	1	11328.591	1875.623	<0.001
Site	83.416	1	83.416	13.796	<0.001*
Season	96.943	1	96.943	16.033	<0.001*
Site*Season	130.460	1	130.460	21.577	<0.001*
Error	217.669	36	6.046		
Total	11857.080	40			
Corrected Total	528.488	39			

3.4 Discussion:

Centrostephanus tenuispinus is known to distributed in wide range of western coast of Australia (Abrolhos Islands - 28°43´S,113°47´E; Fremantle - 32°S) and southern Australia (Spencer Gulf - 34.3036°S, 136.9805°E; Great Australian Bight - 35.7696°S,131.2809°E) in South Australia (Fell 1975). So far, no previous studies have been recorded on the reproductive periodicity of *C. tenuispinus* species in any of these locations. This study provides new information on the reproductive biology of this particular species.

This study revealed the presence of clear synchronized annual reproductive cycle for *C. tenuispinus*, gametogenesis initiating in autumn and spawning in winter. As with the majority of high latitude sea urchins, *C. tenuispinus* also exhibits seasonal spawning. Many species in high latitudes tend to spawn in optimal weather conditions ensuring larval survival (Bronstein et al. 2016). Seasonal reproduction has also been witnessed in other high latitude urchins *Psammechinus miliaris*, *Arbacia lixula*, *Paracentrotus lividus* (Shpigel et al. 2004), *Centrostephanus rodgersii*, *Heliocidaris erythrogramma* and *Evechinus chloroticus* (Brewin et al. 2000; Byrne et al. 1998; Dix 1977; King et al. 1994; Lamare et al. 2002; McShane et al. 1996; Pecorino et al. 2013a; Walker 1982). Reproduction of many high latitude species is influenced by day length or sea water temperature or both (Bronstein et al. 2016; Byrne 1990; Kelly 2001; Pearse et al. 1986). Although there was no correlation between GSI and sea water temperature/day length in this study, histological analysis revealed that reproduction of *C. tenuispinus* is influenced by lower temperatures and short daylight hours. Gametogenesis was initiated with lowering sea water temperature and decreasing day length. Although a few studies suggest that sea water temperature does not play a central role in regulating reproductive cycles in Indo-Pacific sea urchins (Drummond 1991; Pearce and Scheibling 1991), sea water temperature is known to influence reproduction in many high latitude species at different scales (Byrne 1990; Drummond 1995; González-Irusta et al. 2010). Coppard and Campbell (2005b) recorded increased reproductive output of *Diadema savignyi* in response to increasing temperature and in Fiji. Gametogenesis of *Diadema antillarum* in Bermuda is known to favour sea water temperatures above 20°C. Yet, temperatures over 25°C are known to inhabit the gonad growth of *D. antillarum* (Iliffe and Pearse 1982). In contrast, gametogenesis of *Tripneustes gratilla* in Madagascar is influenced by decreasing temperature and short days (Vaitilingon et al. 2005). The majority of high latitude urchins in the northern hemisphere spawn in spring, summer or autumn when the temperature is higher than the rest of the year (Byrne 1990; González-Irusta et al. 2010). Species from southern high latitudes, such as *Evechinus chloroticus* and *Heliocidaris erythrogramma*, have been documented to spawn in spring, summer or autumn (Brewin et al. 2000; Dix 1977; Lamare et al. 2002; Walker 1982). *Hemicentrotus pulcherrimus* in Fukui, Japan spawn in response to decreasing temperature from 13 °C to 10 °C; thus spawning initiates in winter. The same species in south west Japan has delayed spawning in spring, in response to increasing temperature 6 °C - 13 °C. This indicates that this species spawns in a similar range of temperatures despite the location. A few species, such as *C.*

rodgersii in Australia and *Strongylocentrotus purpuratus* in the northern hemisphere, are also known to spawn in winter when the sea water temperature is at its lowest, showing a similar trend to *C. tenuispinus* in the current study (Byrne et al. 1998; King et al. 1994; Ling et al. 2008). This could be related to a long planktonic larval stage.

Diadematoid sea urchins are well known to be impacted by the lunar cycle, mostly spawning at the new moon or full moon (Coppard and Campbell 2005b; Kennedy and Pearse 1975). Spawning of *Centrostephanus rodgersii* in New South Wales is closely related to short day length and lunar cycle corresponding with the winter solstice (Byrne et al. 1998). Monthly sampling was carried out for this study and was not focused on lunar synchronization. But as with other diadematoids, the potential of *C. tenuispinus* spawning in response to lunar cycle should be considered.

The reproductive cycle of *C. tenuispinus* shows similar trends to that of *C. rodgersii*, its congener on the east coast of Australia. The gametogenesis process in both species initiated in March, and similarities in the gonad histological process during gametogenesis were also witnessed during this study (Byrne et al. 1998; King et al. 1994). The cellular process of gonads during these periods was similar to other regular echinoids as well (Byrne 1990; Drummond 1995). *C. tenuispinus* initiated spawning in July-August, while *C. rodgersii* has been recorded to spawn during the winter solstice (June) in New South Wales (Byrne et al. 1998; King et al. 1994). Variations spawning duration (1-5 months) of *C. rodgersii* has been documented over the range of 7° latitudes in New South Wales, leading to prolonged spawning at higher latitudes. *C. tenuispinus* in this study has also exhibited prolonged spawning (5 months), similar to *C. rodgersii* in southern temperate regions of the east coast (Eden 37°S) (Byrne et al. 1998). In contrast to the latitudinal trend observed on the mainland coast of eastern Australia, Tasmanian populations (41°-43°) of *C. rodgersii* have a short spawning season (1 - 2 months) (Ling et al. 2008). Similarly, *C. rodgersii* in New Zealand (36°S) is known to have a shorter spawning period (Pecorino et al. 2013a).

In scenarios where reproduction is restricted to certain parts of the year, reproductive cycles in different populations synchronized as well. *Lytechinus variegatus* indicated greater seasonal synchrony in high latitude populations than near the equator. Reproduction of *C. rodgersii* in northern New South Wales is known to be highly synchronised, yet southern temperate areas of the eastern coast of Australia are weakly synchronized (Byrne et al. 1998). Prolonged spawning in southern temperate regions could also be associated with weak synchronization. Although *C. tenuispinus* exhibit prolonged spawning, their reproductive cycle was well synchronized between sexes at

Hall Bank reef. Reproductive synchrony has also been observed in many other diadematoid sea urchins (Drummond 1995; Pearse 1970).

The interaction of GSI between genders and month/season is observed when gonads of one gender are larger compared the other gender or one gender is capable of spawning more gametes (Grant and Tyler 1983). High GSI values of *C. tenuispinus* recorded prior to spawning indicates that the gonads are full of mature gametes in both males and females at that time. Inconsistency of GSI values for both genders with histological analysis indicates that changes in GSI values alone do not provide proper understating of the reproductive cycle (Byrne et al. 1998). Presence of early developing stages in June and July indicated prolonged spawning in this species. These individuals were responsible for late spawning events (September-December). Gametogenesis of *C. tenuispinus* females (March) initiated one month earlier than males (April). Since female gonads are nutrient reserves for future larvae, these tend to accumulate a lot of nutritive tissue reabsorbing unshed gametes from previous cycle. In most cases non-feeding larval survival depends upon stored energy resources derived from the egg. The short premature stage observed in both genders indicates a short and rapid maturation period. This has been recorded for *C. rodgersii* as well (Pecorino et al. 2013a). Presence of Lipofuscin in gonads indicated the recovery stage. Lipofuscin is known as the "wear and tear pigment", and is responsible for reabsorbing unshed ova/sperms; it is common in gonads in recovery stage (King et al. 1994). Slight differences in the composition of gametogenic stages in December 2014 and 2015 could be attributed to external factors in the environment.

The close relationship between feeding and gonad growth has been recorded in many regular echinoids. Food ingestion is important to gonad growth and gonads are a major nutrient storage organ in sea urchins. Food abundance and nutritive quality of the food directly impact the gonad size and quality (Byrne 1990; Byrne et al. 1998). Similar trends in gametogenesis and spawning for *C. tenuispinus* were recorded for samples collected from Minden Reef. Higher gonad indices of these urchins were attributed to higher food availability at Minden Reef (Chapter 2). On the other hand, low gonad indices in urchins at Hall Bank reef reflect food scarcity in this particular habitat. Differences in gonad growth and quality on fringe areas and barrens is well known in other geographical areas (Byrne et al. 1998). The inverse relationship between Repletion Index (RI, index of gut fullness) and GSI has been recorded in some sea urchin populations. The significant differences recorded for RI are influenced by seasonal variation in food availability as well. Higher RI of *C. tenuispinus* in February

(initiation of gametogenesis) and in September (post spawning) could be attributed to seasonal food availability, and energy requirements pre- and post-reproduction. High RI has been recorded in *Tripneustes gratilla* in Madagascar after spawning and after initiation of gametogenesis (Vaitilingon et al. 2005).

C. rodgersii have shown differences in relation to food availability in Australia and New Zealand. Average GSI for males ranges 7.6 ± 4.4 % to 21.9 ± 3.9 % female 6.8 ± 1.9 % to 16.1 ± 4.4 % for New Zealand population of *C. rodgersii* in Mokohinen Island (Pecorino et al. 2013a). According to Ling et al. (2008), the Tasmanian population has a range of 18.4-20.1 % in GSI, attributed to differences in diets; this has been previously recorded in other high latitude species as well (Meidel and Scheibling (1998). Higher GSI values have been recorded for sheltered subtidal populations of *Paracentrotus lividus,* compared to exposed intertidal populations on the west coast of Ireland (Byrne (1990). *C. tenuispinus* at Minden Reef was larger and had a lower density of urchins compared to Hall Bank reef (Chapter 2). Thus the cumulative impacts of higher population density and food scarcity have been attributed to the decrease in reproductive output in Hall Bank. Similarly, an inverse relationship between population density and gonad size has been documented for *Evechinus choloroticus* in New Zealand.

C. tenuispinus showed omnivory and is known to feed on a considerable amount of animal tissues (Vanderklift et al. 2006) (Chapter 4). High levels of nutrients in their diets due to increased feeding on animal tissues in summer and autumn can also influence the initiation of gametogenesis. Influence of diet on reproductive maturation and growth rate has been demonstrated in previous studies on *Strongylocentrotus* sp. and *Paracentrotus* sp. (Cook and Kelly 2007b; Jacquin et al. 2006; McBride et al. 2004; Meidel and Scheibling 1998). Diets of high quality food in high abundance accelerate not only growth rate and gonad quality but also enhances the survival of juveniles and young adults as well (Meidel and Scheibling 1999). The ability of nutritive phagocytes to respond to food of different quality is used by fisheries and aquaculture to enhance gonad output through control of diet (Cook and Kelly 2007b; McBride et al. 2004; Pearce et al. 2004; Shpigel et al. 2005).

Centrostephanus rodgersii is known to attain a sexual maturity test diameter of 40 mm to 60 mm in Australia and New Zealand (King et al. 1994; Pecorino et al. 2013a). There is no previous data on size at maturity for *C. tenuispinus*. The smallest individual of *C. tenuispinus* observed in the field was 38 mm (Chapter 2). All samples collected were sexually mature and immature samples were not observed. Due to the

lack of smaller indiviuals, estimating the size at sexual maturity for *C. tenuispinus* was not possible in this study.

Many urchin species have a short larval stage before settling. Evidence suggests that temperature plays a major role in larval development (Hart and Scheibling 1988). *Centrostephanus rodgersii* is known to have a long free-swimming larval phase (4 months), before settling down as an adult. The larva is mainly developed through spring during a period of high food abundance, and tends to settle in summer. Since *C. tenuispinus* shares similarities with the reproductive cycle of *C. rodgersii*, development and recruitment of the larval stages have the potential to follow the same patterns as its east coast counterpart. Further, the prolonged planktonic larval stage in *C. rodgersii* has been one of the main reasons for the expansion of its range to New Zealand following the East Australian Current. *C. tenuispinus* being an omnivore, has potential to create barrens (Chapter 4). Thus, knowledge on larval recruitment will also be beneficial for better management and conservation of *C. tenuispinus* dominated reefs.

Chapter 4 - Feeding Ecology of *Centrostephanus tenuispinus*

4.1 Introduction

4.1.1 Sea urchins as grazers in benthic ecosystems

Sea urchins are well known grazers and their herbivorous nature means they are a crucial component in many marine benthic habitats (Byrnes et al. 2013a; Carpenter 1990; Lawrence 1975; Lawrence 2013; Scheibling and Stephenson 1984). They exert an immense impact on their habitat through feeding, and the extent of the impact varies depending on spatial and temporal factors as well as feeding mechanisms of the species. At extreme grazing events, the creation of barrens has been observed in different parts of the world (Byrnes et al. 2013b; Filbee-Dexter and Scheibling 2014; Johnson and Mann 1982; Johnson et al. 2004; Ling et al. 2010; Ling et al. 2015). A conceptual model on barrens formation suggests that variability in urchins' consumptive potential is one of the key drivers of kelp forest dynamics (Byrnes et al. 2013b). Reduction in food per capita is also known to drive the habitats into barren status (Livore and Connell 2012b). Presence of urchins in high densities alone doesn't drive habitats into barrens; the effect of urchins in some areas depended on their feeding biology (Byrnes et al. 2013b). Thus, knowledge on species-specific urchin feeding ecology is essential to assess their impact on habitat.

4.1.2 Sea urchin feeding

Sea urchins are known as generalised herbivores. The composition of their algal diets is known to vary geographically and seasonally depending on food availability and their preferences (Beddingfield and McClintock 1998; Lawrence 1975). Preferences of sea urchins for certain algae species over others are known to depend on nutritional value and defensive mechanisms (calcification and chemical nature) of the algae (Dworjanyn et al. 2007; Klumpp et al. 1993; Konar 2000; Seymour et al. 2013; Stimson et al. 2007; Wright et al. 2005). Urchins inhabiting bare substrate are known to feed on microalgae/turf, while urchins inhabiting many temperate regions are well known for their extensive feeding in kelp (Lyimo et al. 2011; Scheibling 1986; Scheibling et al. 1999). Sea urchins' tendency to feed on animal tissues has also been recorded in many previous studies (Bak and van Eys 1975; Jacob et al. 2003; Lewis 1964; Vanderklift et al. 2006).

Sea urchins are active grazers and capable of changing their feeding habits in response to food availability in respective habitat. The Aristotle's lantern is a highly

specialised masticatory apparatus, composed of five pyramidal structures and five mineralised teeth which is used in their grazing (Ma et al. 2008). Teeth are continuously formed at the distal end of the lantern and mineralised towards the oral end. Muscles are used for movement of lantern teeth when they are feeding.

Sea urchins can be categorised into two groups based on feeding mechanisms: active grazers are those that scrape attached forms, and drift feeders are those that capture drifting algae with their tube feet. Presence of long tube feet have been recorded for many species adapted to feeding on drifting algae (Vanderklift et al. 2009; Vanderklift and Wernberg 2008). Urchins exhibiting benthic feeding are active scrapers that scrape down everything to the substrate and they are known to have strong lanterns (Ling et al. 2010; Wright et al. 2005). Urchins are capable of changing lantern size depending on food availability. Lanterns tend to be relatively larger compared to test size when food is scarce. Thus, the relationship between lantern pyramid length and test size reflects food availability (Ebert et al. 2014). Further, enlarged lanterns have been observed in sea urchins species which feed on hard-shelled prey (Hagen 2008).

4.1.3 Sea urchins feeding rates

Sea urchin feeding depends upon on many factors: food availability, urchin density, environmental conditions and sometimes reproductive state.Urchin density is known to be a key driver for altering urchin feeding behaviours (Steneck et al. 2002). Environmental conditions such as waves can create gaps in kelp forests and restrict urchins feeding as well (Harrold and Reed 1985; Kawamata 1998; Lissner 1980). In some tropical locations *Tripneustes gratilla* is known to feed on seagrass when the habitat is calm; on the other hand, the same species in Hawaii is known to mostly feed in the surf/splash zone (Stimson et al. 2007; Vaïtilingon et al. 2003). Higher urchin feeding rates have been observed in sheltered bays (less food availability) and lower feeding rates in exposed bays (high availability) (Livore and Connell 2012a). These results suggest that food source and availability could help to explain the impacts of these urchins on sheltered coasts, while on exposed coasts other factors such as wave energy and sea urchin density may also contribute to extent of the herbivory (Livore and Connell 2012a).

4.1.4 Sea urchins in marine food webs

Sea urchin food preferences, feeding mechanisms and behaviours have been studied in different localities due to their ecological and economical importance. Gut contents and faecal matter analysis have been used to predict their diet in many past

studies. Due to difficulties in identifying digested or partially digested food particles and difficulties in assessing the trophic position of a species, these conventional methods are becoming less used (Phillips et al. 2014).

Stable isotope analysis is very useful in analysing food web dynamics in an ecosystem (Davis et al. 2012; Fry 2006; Lepoint et al. 2004). Most trophic studies are typically based on analysis of $\delta^{13}C$ and $\delta^{15}N$. High trophic level values indicate the importance of other animal tissues for their diet (Rodríguez-Barreras et al. 2015a). These techniques are used to predict the diet over an extended period of time, as well as on organisms with empty guts. Analysis of stable isotopes hasbeen used in many food web studies in the recent past (Vanderklift et al. 2006).

Urchins that are generalist herbivores are in lower trophic levels. A stable isotope study on *Diadema antillarum* reported that trophic level values ranged from 2.35 ± 0.11 to 3.24 ± 0.17. Similarly, co-existing urchins *Arbacia lixula* and *Paracentrotus lividus* in the Mediterranean are known to occupy different trophic levels. The presence of animal tissues (Cirripedia, Hydrozoa, Bryozoa) in *A. lixula* diets has confirmed their omnivorous nature, which places them in a higher trophic level than *P. Lividus* (Wangensteen et al. 2011).

4.1.5 *Centrostephanus tenuispinus* in Hall Bank reef

In contrast to its congener *Centrostephanus rodgersii* on the east coast of Australia, *C. tenuispinus* is understudied, and its feeding ecology is poorly understood. The only study on *C. tenuispinus* feeding recorded a high percentage of sand and rock fragments (9.5 ± 2.7%) in their gut, compared to other coexisting urchins in macroalgae dominated reefs (*Phyllacanthus irregularis*- 3.7 ± 1.4%; *Heliocidaris erythrogramma*- 0.8 ± 0.2%) (Vanderklift et al. 2006). Further, considerably higher percentage of animal tissues has also been recorded in *C. tenuispinus* gut (10.9 %). Stable isotope analysis of lantern muscles revealed that *C. tenuispinus* is in a higher trophic level compared to the co-existing herbivore *Heliocidaris erythrogramma* (Vanderklift et al. 2006). Outcomes of this study reflect the similarities *C. tenuispinus* feeding behaviours to its congener in eastern Australia. Considering the immense impact of *C. rodgersii* on the east coast of Australia and its newly established habitats, knowledge on feeding ecology of *C. tenuispinus* is essential for future management of its habitats.

The main objective of this chapter is to assess differences in trophic level occupied by *C. tenuispinus* in response to variations in food availability. Further, this study assessed temporal variation in diet composition of *C. tenuispinus* in Hall Bank

reef. Gut composition and trophic level values were compared with urchins from macroalgae-dominated (high food abundant) Minden Reef.

4.2 Methodology

4.2.1 Study site:

The studywas carried out at two study sites: Hall Bank reef (32°2.002´S and 115°42.957´) and Minden Reef (32º04.320′S and 115º 43.782′E) (Chapter 2).

4.2.2 Sampling / Laboratory Procedures

4.2.2.1 Analysis of diet composition of *C. tenuispinus* in Hall Bank reef

Samples were collected at monthly intervals from December 2014 to December 2015. Ten sea urchins were collected per month using SCUBA. Samples were transferred on ice to the laboratory for processing. All samples were blotted dry before recording measurements. Total weight (balance ± 0.001g) and test diameter (Vernier calliper ± 0.1mm) of each sample were measured. Spines were removed, and urchins were dissected to extract the gut. The total gut weight was measured, and a 1cm portion of the gut was removed and preserved in 70% ethanol for later analysis. The remaining gut content was removed from the gut and was dried at 60 °C until it reached a constant weight.

The preserved gut contents were removed from the gut and placed in a beaker and diluted up to 150 mL. Equal portions of the gut content were divided into two Petri dishes, and a mesh (1 X 1 cm grid) was overlaid on each Petri dish. Five 1 X 1 cm squares were haphazardly selected from each Petri dish for analysis. Each square was observed under a dissecting microscope (model: Olympus SZ61), and the frequency of each gut component was recorded. Gut components were identified to genus level where possible; animal components were identified to class level since few segments of the animals were observed. All gut components were photographed with digital cameras Olympus SXY-M90 and Olympus DP70 and associated software.

4.2.2.2 Gut composition of *C. tenuispinus* in Hall Bank reef vs Minden Reef

Ten sea urchins were collected in winter 2016 and summer 2017 from Hall Bank reef and Minden Reef using SCUBA. Samples were transferred on ice to the laboratory for processing. Samples were processed using the same methods as outlined in section 4.2.2.1.The repletion index was calculated separately for each site and season (Chapter 3).

4.2.2.3 Analysis of stable isotopes

Five urchin samples were collected using SCUBA from Hall Bank reef and Minden Reef in winter 2016 and summer 2017 (total of 20 urchins). Algae and other benthic invertebrate species within a 1 m radius of the collected urchins were also sampled (3 samples per species). Samples were transferred on ice to the laboratory for processing. All urchin samples were blotted dry before taking measurements. The sea urchins were dissected, and five Aristotle's lantern muscles were extracted using sterilised forceps, and thoroughly washed with deionised water. Samples were labelled and frozen (-20 °C) for stable isotope analysis.

Turf algae were thoroughly washed with deionised water and separated into broad taxonomic categories under a dissecting microscope. Algae and other animal samples were thoroughly washed with deionised water. All epiphytes were removed from macroalgae using a clean brush. A 1 cm portion of animal samples was cut with a sterilised pair of scissors, labelled and frozen (-20 °C) for stable isotope analysis.

Frozen samples were transferred to a freeze drier (LOBCOHCO 7670530) and freeze dried for 96 hours until samples reached constant weight. Samples were ground in a mixer mill (Mill Mix 400 / Restch) for 20 minutes, and the powdered samples were sent to the West Australian Biochemistry Centre for analysis of stable isotopes. Calcified samples were acidified before analysis. Samples were analysed for $\delta^{13}C$ and $\delta^{15}N$, using a continuous flow system consisting of a Delta V Plus mass spectrometer connected with a Thermo flush 1112 via Conflo (Thermo-Finnigan-Germany).

4.2.2.4 Determination of feeding and grazing rates

The amount of both organic and inorganic material ingested was considered as total ingestion. Total ingestion was used to calculate daily ingestion rates. The amount of organic material ingested (i.e. algae and animal tissues) was used to calculate grazing rate. Seasonal and monthly ingestion and grazing rates of *C. tenuispinus* in Hall Bank were compared using samples collected for bio-erosion (Chapter 5).

4.2.3 Statistical analysis

4.2.3.1 Analysis of diet composition

Each diet component was categorised in to the broader taxonomic group. Frequencies of each diet component were calculated. Averaged data for each month were used for data analysis. Seasonal and site differences in food ingestion rates and grazing rates were analysed using two-way ANOVA using SPSS 24 software. All assumptions for running an ANOVA were met.

Similarities in diet composition between months/seasons were analysed using ANOSIM and PERMANOVA (Primer v6 multivariate software). Data were square-root transformed, and Bray-Curtis similarity matrices were constructed for temporal data from Hall Bank reef. Seasonal data for Hall Bank and Minden Reef were used to represent two-way crossed design of the site (2 levels) and season (2 levels), with each factor being fixed. PERMANOVA was tested on this design to check the possibilities of interactions and level of interaction between these factors. The matrix from Hall Bank temporal dietary data was subjected to one-way ANOSIM, with month and season as factors separately. A dietary matrix created from data from Hall Bank and Minden Reef was subjected to two-way ANOSIM testing for the site, season and combined factors. R statistics and associated p values were used to interpret the output.

A SIMPER analysis was also used to determine the most typical dietary item in each month. Similarities in diet composition between seasons and sites (Hall Bank reef and Minden Reef) were analyzed using Primer v6.

4.2.3.2 Analysis of diet composition using stable isotopes

Trophic position (TP) was calculated using $\delta^{15}N$ values of diet sources and urchins. Since the literature reveals that $\delta^{15}N$ varies both spatially and temporally (Vanderklift et al. 2006), an average of macro- and microalgae present was used for calculation of each study site and season. Differences between $\delta^{15}N$ in sea urchins and baseline $\delta^{15}N$ ($\Delta_{urchin\ –\ macro\ and\ microalgae}$) were analysed by two-way ANOVA for site and season.

Nitrogen enrichment for one trophic level was considered as 1.6, and the trophic position was calculated using the following equation (Vanderklift et al. 2006; Vanderklift and Ponsard 2003):

$$TP = \frac{Sea\ urchin\ \delta 15\mathrm{N} - Algae\ \delta 15\mathrm{N}}{1.6} + 1$$

4.3 Results

4.3.1 Temporal variation of urchin diets in Hall Bank reef

4.3.1.1 Seasonal variation in sea urchin diets

The diet of *Centrostephanus tenuispinus* in Hall Bank reef was composed of calcareous substrate particles, sand and organic materials. Seasonal variation in

inorganic and organic gut content composition in Hall Bank reef is presented in Chapter 5.

The organic component in these sea urchin diets was algae and animal material. The highest percentage frequency of algae material (70.98 ± 1.65%) (mean ± SE) and lowest percentage frequency of animal material (29.02 ± 1.65%) were recorded for Spring 2015 (Figure 4.1). The highest percentage of animal tissues (39.60 ± 5.74%) and lowest percentage of algae tissues (60.40 ± 2.90%) were recorded in summer 2016 (Figure 4.1).

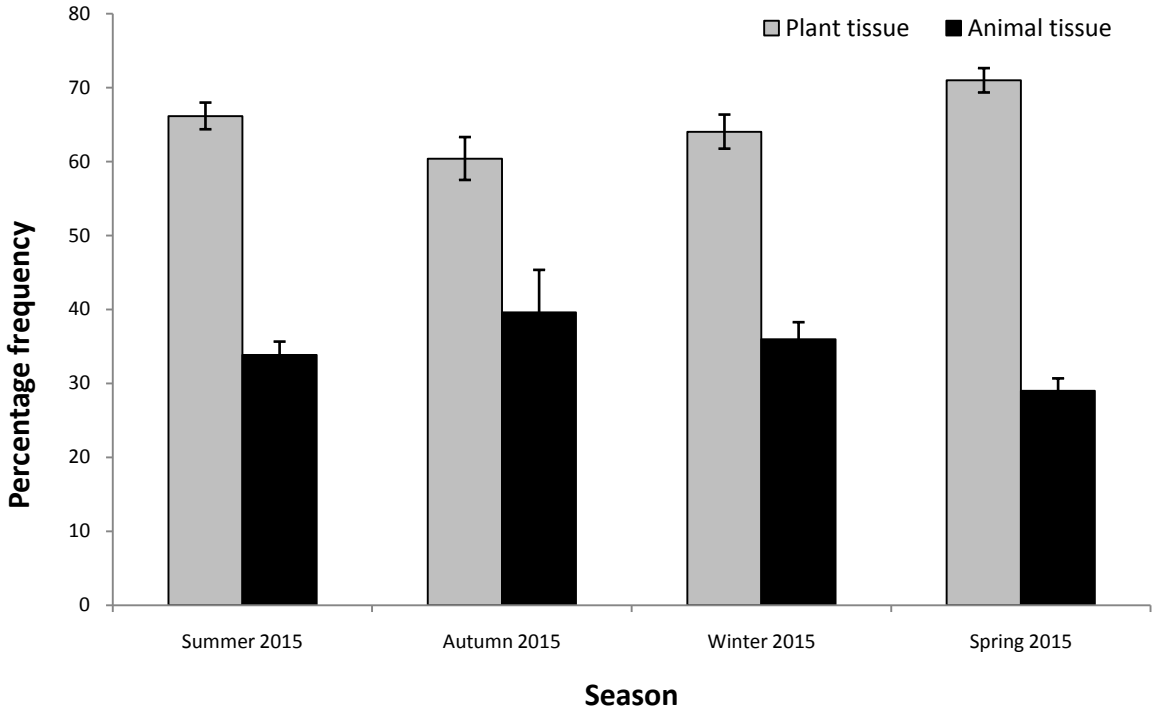

Figure 4.1 Seasonal variation of percentage frequency (mean ± SE) of algae (grey bars) and animal components (black bars) in *C. tenuispinus* diet in Hall Bank reef (n = 30 per season).

Turf algae were the most abundant dietary component of *C. tenuispinus* in Hall Bank reef year round. Turf algae consisted of cropped filamentous algae red, green and brown filamentous algae and blue green algae. The highest seagrass percentage (2.16 ± 0.45%) and lowest macroalgae percentage (0.18 ± 0.11%) was observed in winter (Figure 4.2).

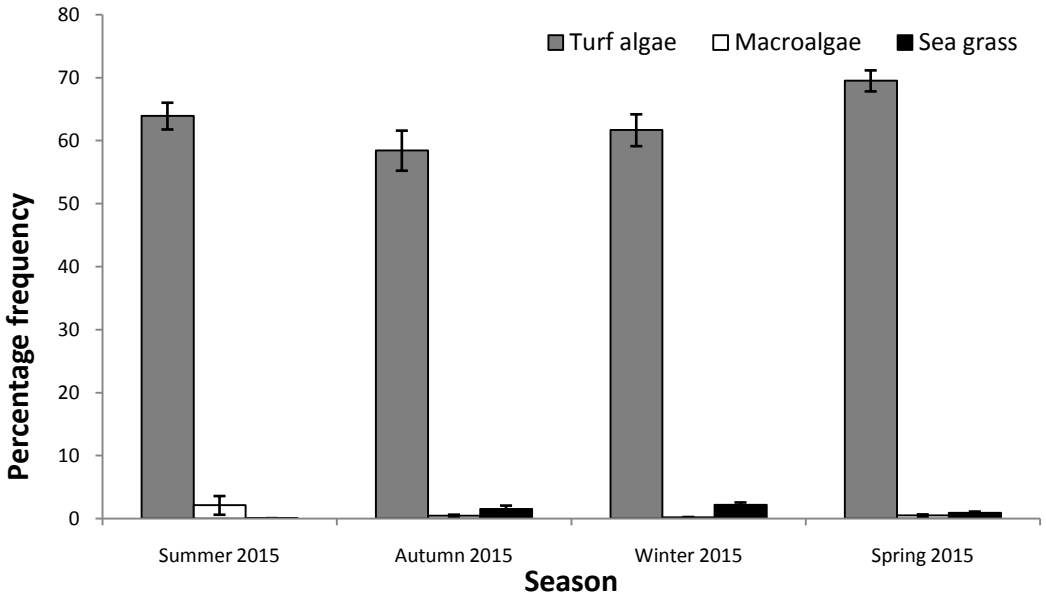

Figure 4.2 Seasonal variation of percentage frequency (mean ± SE) of turf algae (grey bars) and macroalgae (white bars) and seagrass (black bars) in *C. tenuispinus* diet in Hall Bank reef (n = 110).

Diet composition in Hall Bank reef was less variable between seasons (one-way ANOSIM: R = 0.11, p = 0.01%). Diet composition was significantly different only between summer and winter (p = 0.1%) and summer and spring (p = 0.1%) (Figure 4.3).

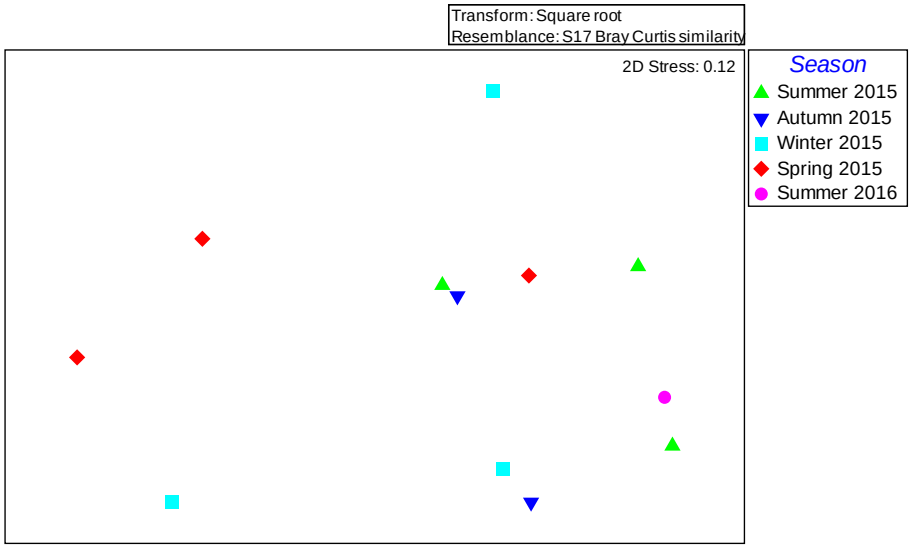

Figure 4.3 nMDS ordination plots, constructed from Bray-Curtis matrices of seasonal percentage frequency of the various dietary items of *C. tenuispinus* in Hall Bank reef.

A SIMPER analysis demonstrated main contributors for the diets in all seasons were turf algae spicules and foraminiferans (Table 4.1).

Table 4.1 Contribution of diet components from SIMPER analysis for *C. tenuispinus* diets in Hall Bank reef from summer 2015-summer 2016 (n = 30 per season).

Diet component	Season	Percentage contribution
Turf algae	Summer 2015	33.54
	Autumn 2015	30.82
	Winter 2015	36.51
	Spring 2015	37.35
	Summer 2016	25.60
Spicules	Summer 2015	19.78
	Autumn 2015	18.96
	Winter 2015	22.03
	Spring 2015	19.15
	Summer 2016	16.69
Foraminiferans	Summer 2015	9.94
	Autumn 2015	13.50
	Winter 2015	10.80
	Spring 2015	11.06
	Summer 2016	12.14

Seasonal mean frequency of red turf algae, green turf algae, brown turf algae, macro algae, seagrass and Cyanobacteriawere 53.18 ± 1.73%, 4.23 ± 1.39%, 2.09± 0.55%, 0.92 ± 0.43%, 1.38 ± 0.53% and 3.19 ± 1.61% (mean ± SE) respectively (Figure 4.4). Green turf algae consisted of *Ulva* (0.19 ± 0.08%) and *Cladophora* sp. (4.04 ± 1.37%) (Table 4.2). *Sphacelaria* sp., *Feldmannia mitchelliae* and *Padina* sp. represented the brown algae. The most abundant turf algae were red turf algae, supporting the outcome of SIMPER.

Polysiphonia sp. was the most abundant dietary item of these urchins in Hall Bank reef irrespective of the season (Table 4.2; Figure 4.5), contributing over 40% of the *C. tenuispinus* diet. *Sphacelaria* sp. (0.68±0.50%) was only present in autumn 2015, and *Scytosiphon* sp. (0.02 ± 0.02%) was only present in summer 2015. *Polysiphonia* sp. (51.62±3.37%), *Ceramium* sp. (6.49±1.55%) and *Lyngbya* sp. (7.76±1.79%) had the highest percentages in spring.

The number of dietary items recorded in summer 2015, autumn 2015, winter 2015 and spring 2015 were19, 18, 17 and 17 respectively. A total of 21 dietary items were recorded in Hall Bank reef from 2014 - 2015 (Table 4.2).

Table 4.2 Percentage frequency of dietary components in *C. tenuispinus* gut from summer 2015-spring 2015 (mean ± SE), (n = 30 per season).

Phylum	Species/Common name	Summer 2015	Autumn 2015	Winter 2015	Spring 2015
Magnoliophyta	Seagrass	0.07±0.04	1.50±0.61	2.16 ±0.45	0.94±0.22
Chlorophyta	*Enteromorpha* sp.	0.17±0.07	0.39 ±0.2	0.20±0.11	
	Cladophora sp.	7.91±1.45	4.08±1.07	2.43±0.72	1.75±0.57
Rhodophyta	*Polysiphonia* sp.	46.21±2.55	44.94±2.84	49.73±2.07	51.63±3.38
	Ceramium sp.	5.71± 0.86	5.14± 0.95	2.88± 0.57	6.49± 1.55
	Scytosiphon sp.	0.02± 0.02			
Ochrophyta	*Sphacelaria* sp.		0.68±0.50		
	Feldmannia mitchelliae	2.45±0.56	2.36±0.61	3.35±0.75	1.89±0.82
	Padina sp.	1.45±1.44			0.33±0.16
	Other spp.	0.69±0.32	0.44±0.22	0.18±0.11	0.17±0.15
Cyanophyta	*Lyngbya* sp.	1.48±0.99	0.85±0.69	3.10±0.95	7.76±1.80
Sarcomastigophora	Foraminiferans	6.18±0.82	9.19±0.83	5.51±0.7	5.05±0.65
Bryozoa	Bryozoan	1.38±0.30	2.42±0.67	1.60±0.40	0.56±0.20
Porifera	Sponges	0.53±0.23	0.06±0.06	0.03±0.03	0.04±0.04
	Spicules	18.63±1.46	17.37±1.47	20.69±1.60	16.55±1.21
Cnidaria	Colonial Hydrozoa	0.73±0.26	0.42±0.22	0.55±0.28	0.92±0.38
Annelida	Polychaete	0.30±0.15	1.88±0.71	1.38±0.32	1.04±0.30
Mollusca	Gastropods	2.15±0.35	2.75±0.52	1.26±0.31	0.97±0.24
	Bivalve	0.85±0.22	1.15±0.55	1.15±0.23	0.66±0.24
Arthropoda	Arthropods	3.08±0.44	4.36±0.71	4.00±0.58	3.22±0.58
Number of prey items		19	18	17	17

Animal tissues recorded in *C. tenuispinus* gut were comprised of spicules (18.31 ±0.09%), foraminiferans (6.48 ± 0.93%), arthropods (3.66 ±0.30%), polychaetes (1.15 ±0.33%), gastropods (1.79 ± 0.40%), bryozoans (1.49 ± 0.38%), bivalves (0.95 ± 0.11%), colonial hydrozoans (0.60 ± 0.13%) and poriferans (sponges) (0.17 ± 0.12%) (seasonal mean ± SE) (Figure 4.4/4.6). The most frequently observed animal component was spicules followed by foraminiferans and arthropods (Figure 4.7). Spicules (20.69 ± 1.60%) and colonial hydrozoans (0.92 ± 0.38%) were highly abundant in the gut of *C. tenuispinus* in winter. Poriferans were highest in summer (0.63 ± 0.23%). All other animal tissues were observed in highest percentages in autumn.

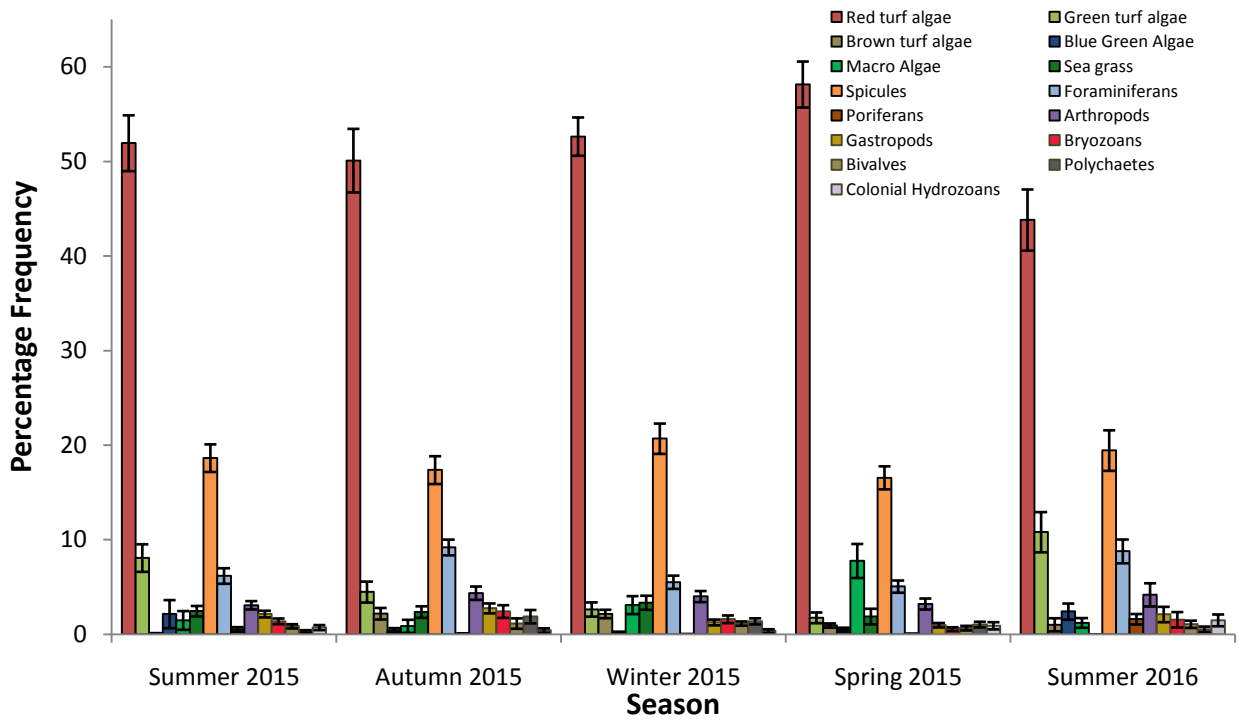

Figure 4.4 Seasonal variation of dietary items in *C. tenuispinus* gut in Hall Bank Reef (n = 30 per season) (mean ± SE).

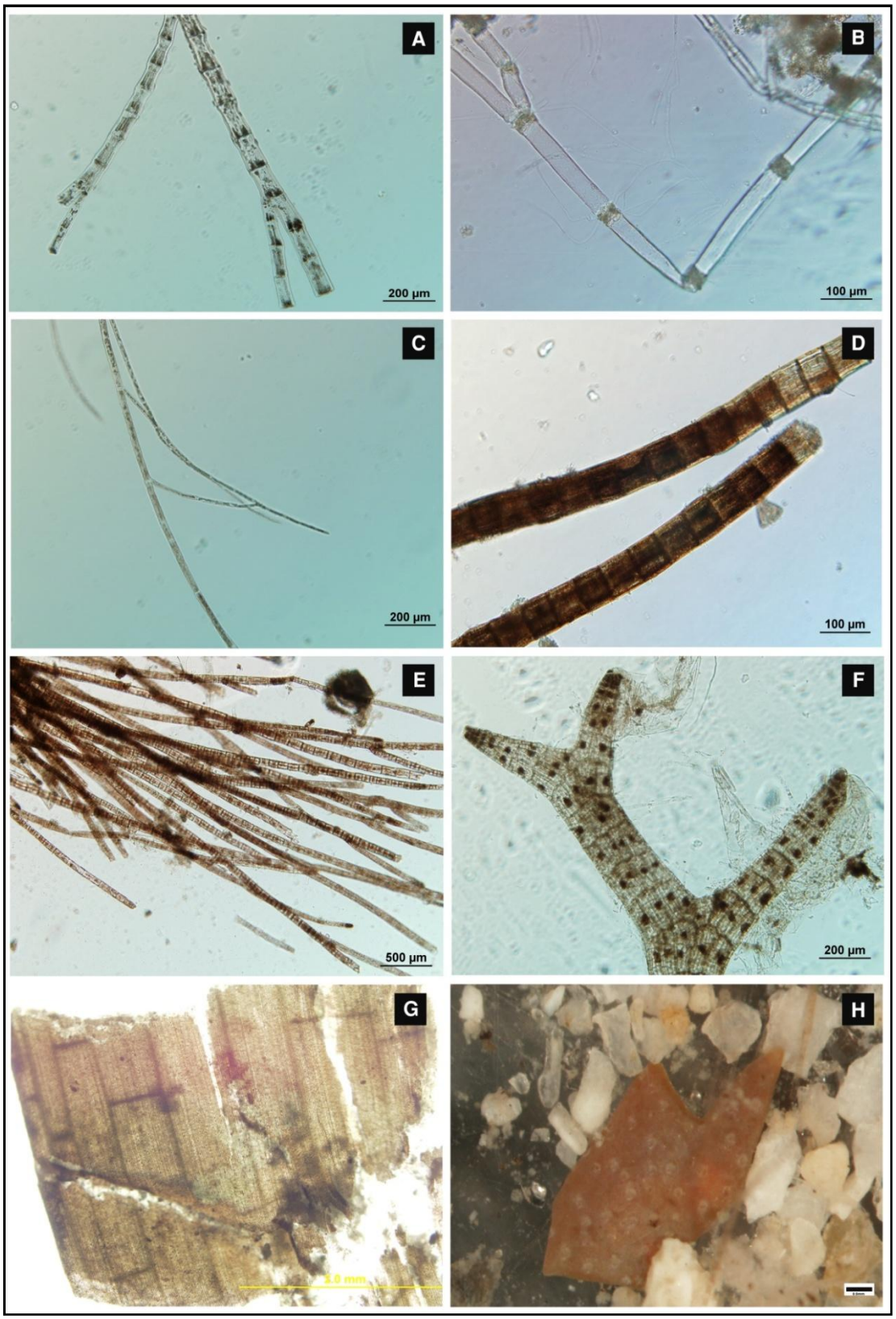

Figure 4.5 Images of different species of algae tissues present in diets of *C. tenuispinus* at Hall Bank reef (A: *Polysiphonia* sp., B: *Ceramium* sp., C: *Cladophora* sp., D - E: *Sphacelaria* sp., F: *Padina* growth stage, G: Seagrass, H: hard substrate particles.

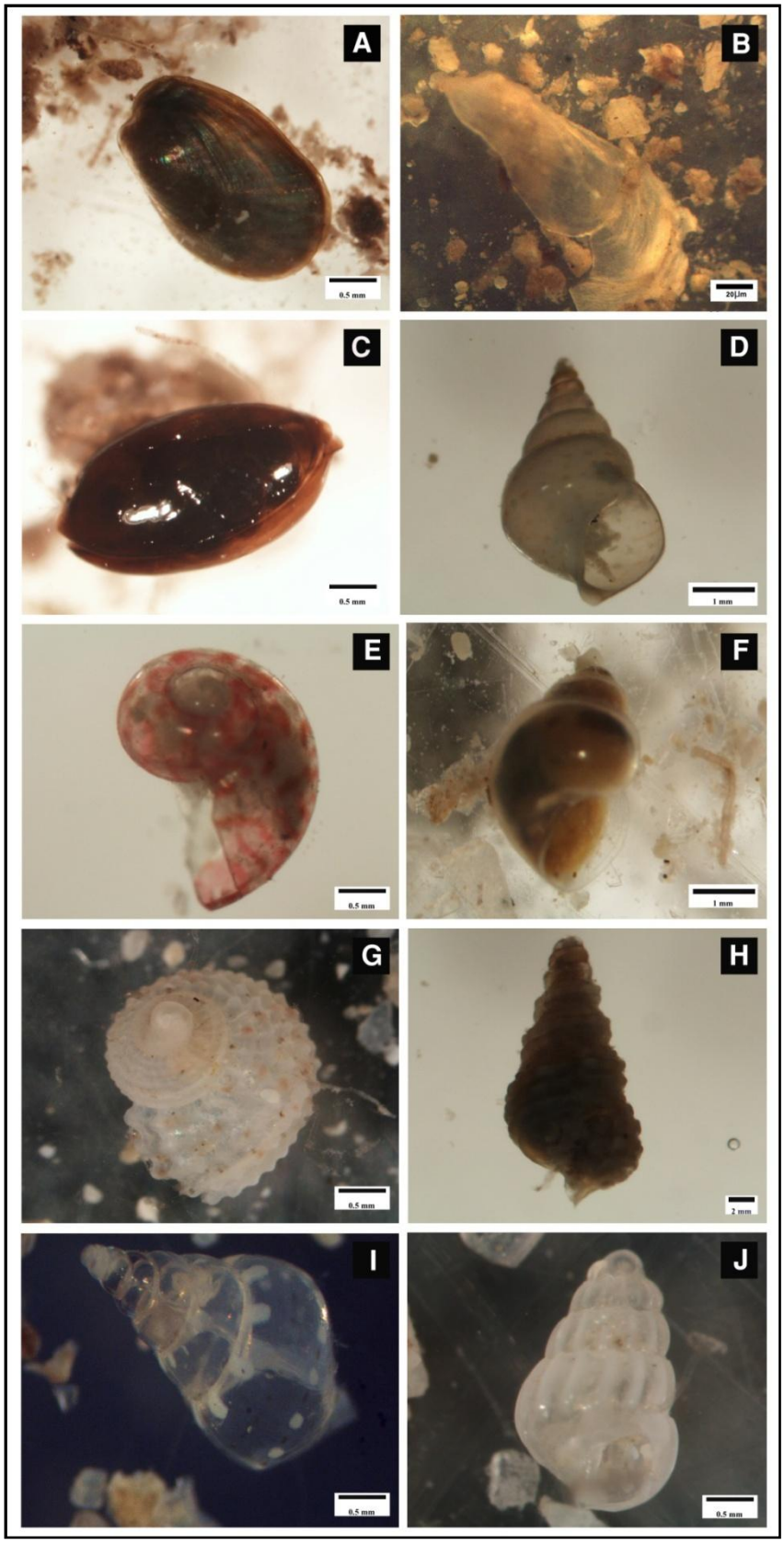

Figure 4.6 Images of different species of animal tissues present in diets of *C. tenuispinus* Hall Bank reef (A - C: bivalves, D-J: gastropods).

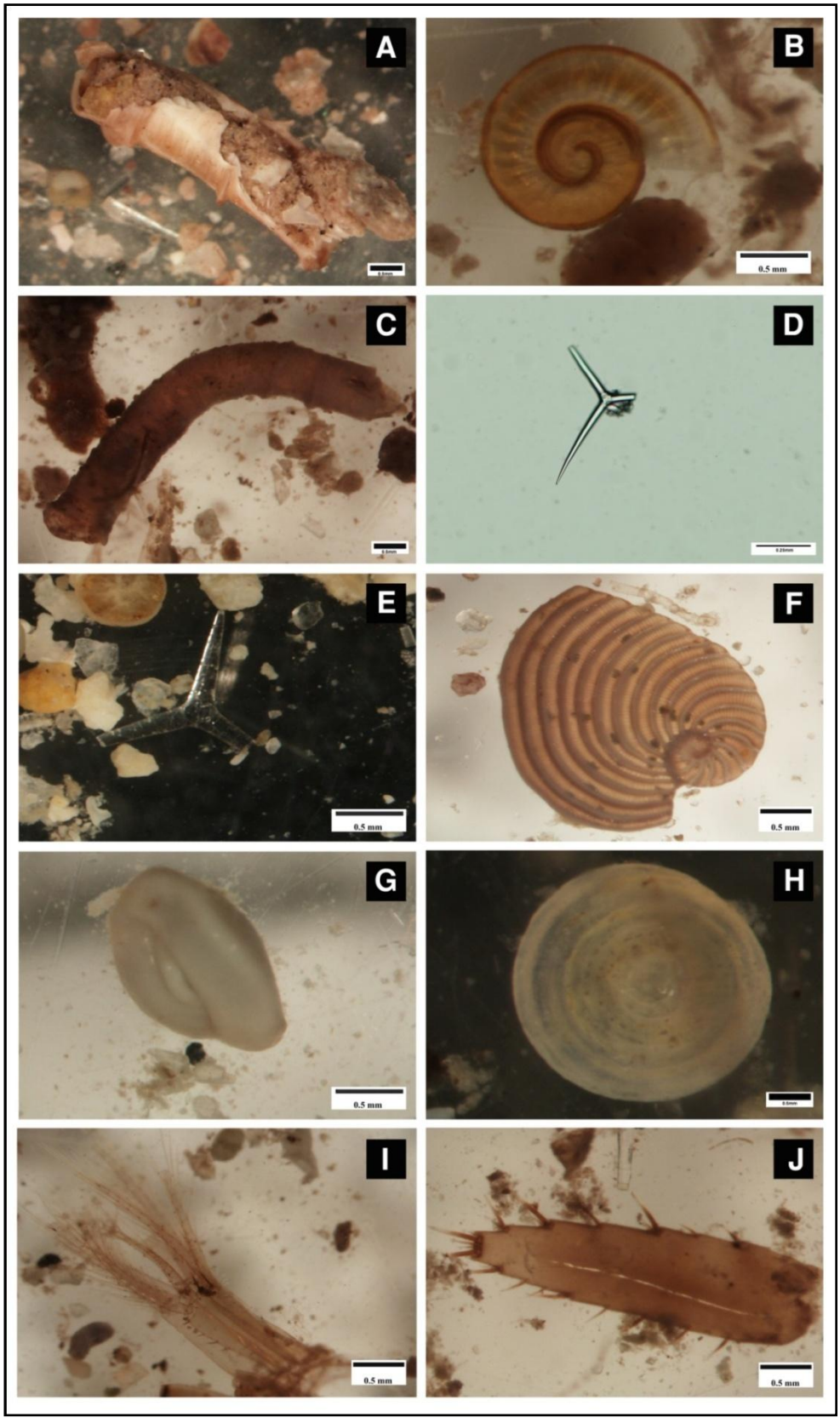

Figure 4.7 Images of different species of animal tissues present in diets of *C. tenuispinus* Hall Bank reef (A-C: polychaete tubes, D-E: spicules, F-H: foraminiferans, I-J: arthropod tissues).

In summary, red microalgae and spicules were the most abundant dietary items in urchin's gut all year in Hall Bank reef. In addition, green microalgae and arthropods were highly abundant in summer and autumn compared to other two seasons (Figure 4.4).

4.3.1.2 Monthly variation in sea urchin diets

A one-way ANOSIM conducted for monthly differences in diets revealed that diet composition was significantly variable between months (R = 0.45, p = 0.1%) (Figure 4.8). Similar to outcomes from the seasonal analysis, SIMPER analysis of monthly diet composition revealed that highest percentage contribution for gut contents was from red turf algae and spicules in all months.

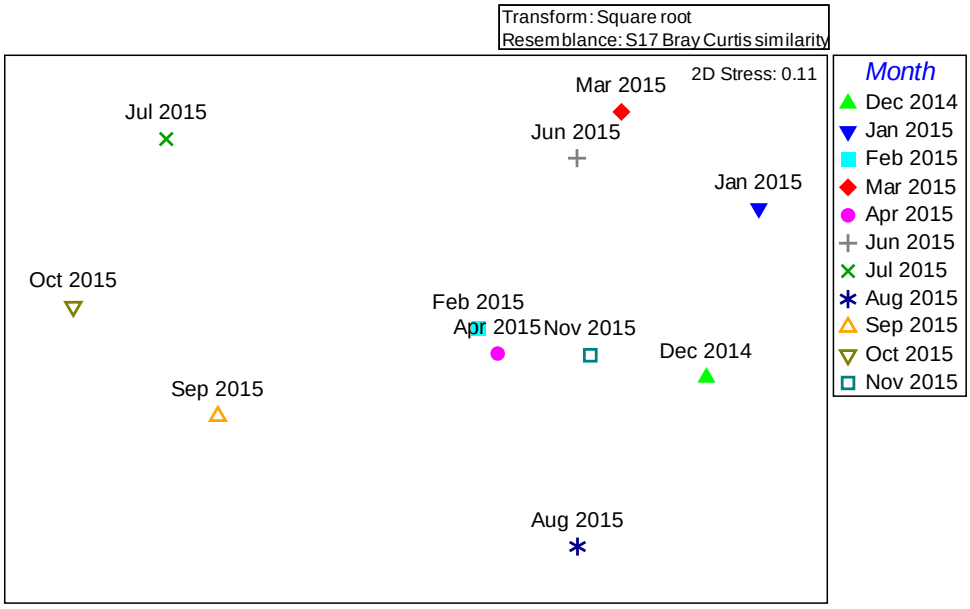

Figure 4.8 nMDS ordination plots, derived from the distance between centroid matrices constructed from Bray-Curtis matrices of percentage mean monthly frequency of diet composition in Hall Bank reef.

Foraminiferans contributed considerably to the diets in February 2015, March 2015, April 2015, June 2015 and September 2015. Green turf algae contribute for December 2014 (11.32%), January 2015 (14.43%), August 2015 (11.25%), November 2015 (8.77%) and December 2015 (12.14%). Cyanobacteria contribute to the diet of July (12.41%) and October (17.79 %) considerably.

Similar to seasonal analysis, the highest contributor to the *C. tenuispinus* diet was red turf algae (52.66 ± 2.75%) (monthly mean ± SE) (Figure 4.9). The highest percentages of red turf algae were recorded in February (64.08 ± 2.09%) and September (64.95 ± 4.06%), while lowest percentages were recorded in January (40.60 ± 2.09%).

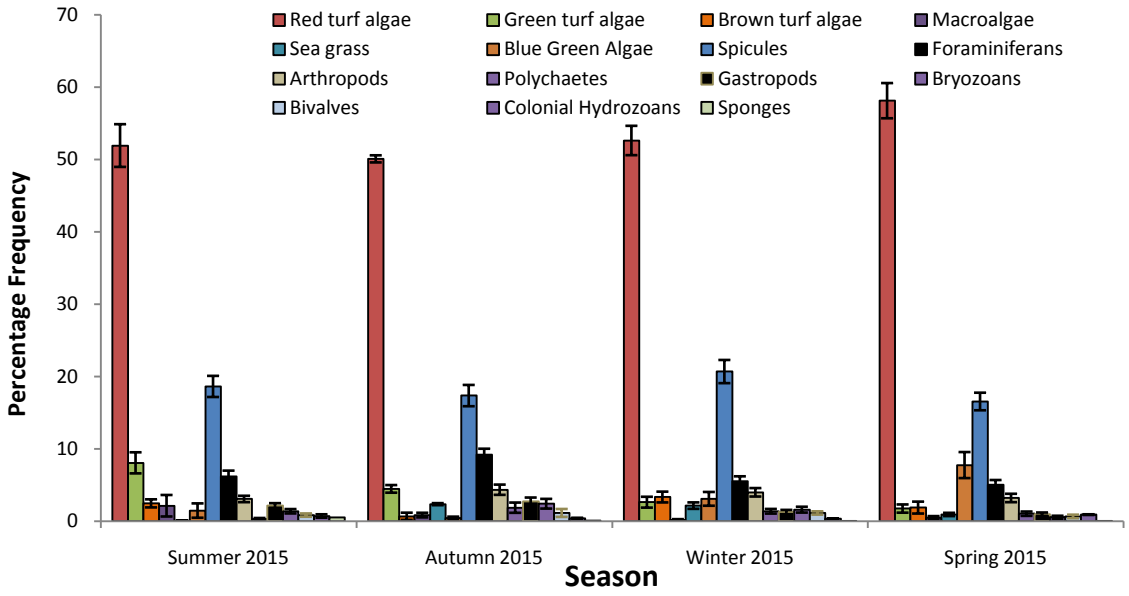

Figure 4.9 Monthly variation of percentage frequency (mean ± SE) of diet components in *C .tenuispinus* diet in Hall Bank reef (n = 30 per season).

The frequency of spicules present in urchin diet was over 15 % in all months (Figure 4.8). A few species of bivalve and gastropod shell were also recorded in their diets. The highest frequency of spicules (22.13 ± 3.88%) was observed in June 2015. Foraminiferans (9.30 ± 1.52%), bryozoans (4.80 ± 0.80%), polychaetes (1.88 ± 0.71%), gastropods (3.88 ± 0.85%) and arthropods (5.08 ± 0.74%) were most abundant in urchin diets from March. Colonial hydrozoans and poriferans were the least abundant dietary items. Spicules, foraminiferans and arthropods were present all year. All animal components (9) were recorded from December to June (except April). Six and five prey items were recorded for August and September respectively.

4.3.2 *Centrostephanus tenuispinus* feeding; Hall Bank reef vs Minden Reef

4.3.2.1 Comparison between gut composition of *C. tenuispinus* in Halls bank and Minden Reefs

Minden Reef urchins had higher dry gut weights compared to Hall Bank urchins ($F_{(1,40)} = 8.25$, $p = 0.007$). No seasonal differences were observed in dry gut weights between the two sites ($F_{(1,40)} = 2.76$, $p = 0.105$) (Table 4.3). Hall Bank urchins had a significantly higher percentage of calcium carbonate (88.50 ± 0.31%) (mean ± SE) ($p < 0.001$) and lower percentage of organic component(8.43 ± 0.20%) ($p = 0.000$) and other siliceous materials (3.06 ± 0.30%) ($p = 0.010$) than Minden Reef urchins. No seasonal differences were recorded in these components for both study sites (Figure 4.10).

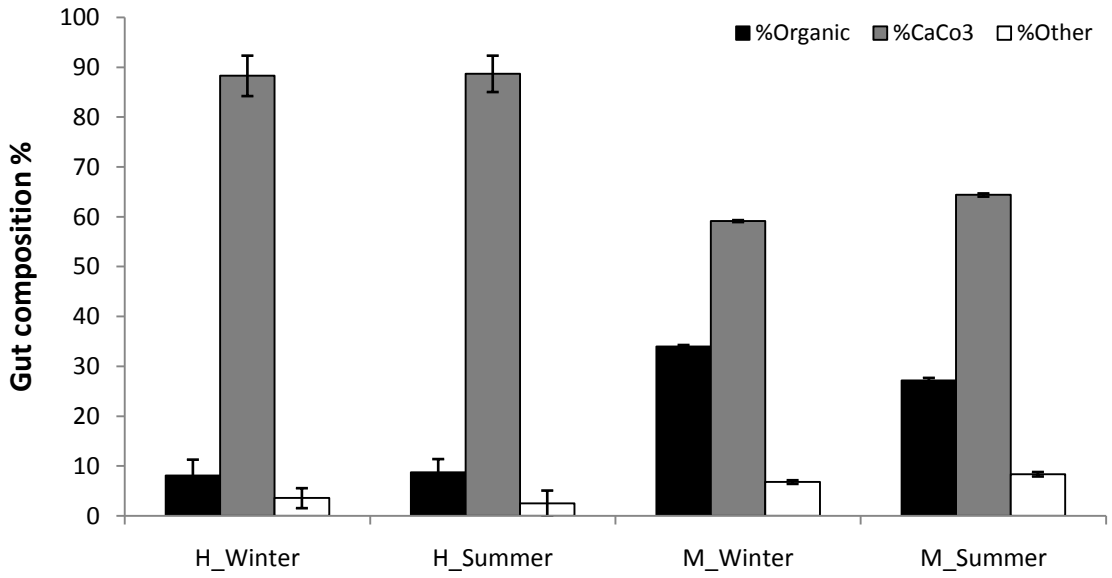

Figure 4.10 Gut composition of *C. tenuispinus* (mean ± SE) in Halls bank reef and Minden Reef; organic component (black bars), CaCO3 (grey bars), siliceous (White white bars) (n = 40).

Hall Bank urchins (summer = 46.07± 4.63%, winter = 27.44 ± 0.98%), had more animal tissues in their diet compared to Minden Reef urchins (winter = 10.47 ± 2.35%, summer = 16.22 ± 2.55%) (Figure 4.11).

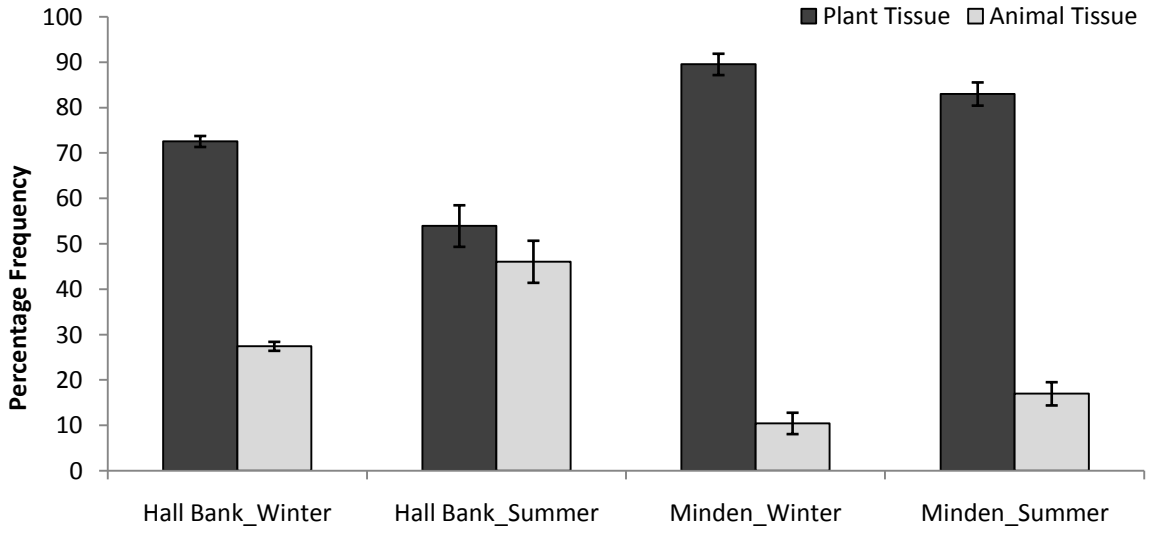

Figure 4.11 Monthly variation of percentage frequency (mean ± SE) of algae (grey bars) and animal tissues (black bars) in *C. tenuispinus* diet in Hall Bank reef (n = 110).

The repletion index for urchins at Hall Bank reef was 15.63 ± 0.67% and 15.13 ± 0.57% in summer and winter respectively. Urchins at Minden Reef had a higher repletion index in winter (21.63 ± 0.98%) than in summer (14.91±0.81%).

The algae tissues in the urchin diet mainly were composed of microalgae, macroalgae and seagrass in both sites. The diet of *C. tenuipinus* in Hall Bank was mainly composed of turf algae in summer (43.89 ± 6.92%) and winter (56.10 ± 1.93 %), while in Minden Reef urchin diets were mainly composed of macroalgae in winter (64.63 ± 2.61 %) and turf algae in summer (41.13 ± 4.87%) (Figure 4.12).

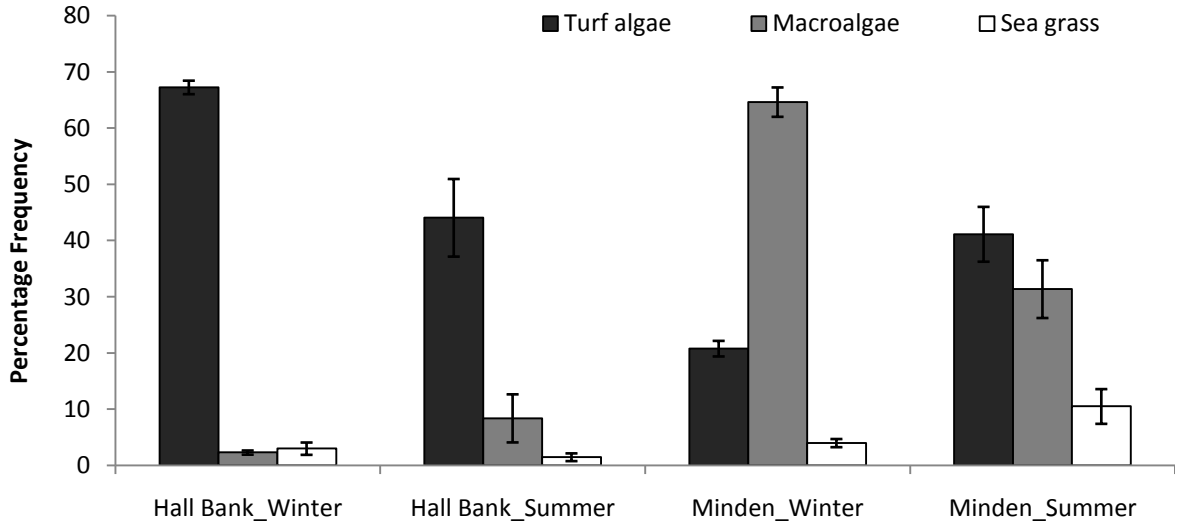

Figure 4.12 Variation of algae tissues (microalgae, macroalgae and seagrass represented by black bars, grey bars and white bars respectively) in *C. tenuispinus* diet in Hall Bank reef and Minden Reef in winter and summer (n = 40) (mean ± SE).

As the results of PERMANOVA suggest, diet composition varies significantly between the two sites and seasons as well. Further, the two-way interaction between sites and season was also significant (p = 0.001). The coefficient of variation (COV) was greater for the site factor (Table 4.3). A two-way crossed ANOSIM revealed the significant differences in diet composition between sites (R = 0.851, p = 0.1%), and seasons (R = 0.5, p = 0.1%). A clear separation was apparent between sites and seasons as well (Figure 4.13).

Table 4.3 Mean squares (MS), pseudo F-ratios, coefficient of variation (COV) and significance level (P%) for site x season, PERMANOVAs for the Bray-Curtis matrices of the percentage frequency dietary items of *C. tenuispinus* Hall Bank reef and Minden Reef.

Source	df	MS	Pseudo-F	COV	P%
Site	1	30787.00	47.511	1507.00	0.001
Season	1	4770.60	7.3619	206.13	0.001
Site X Season	1	4597.60	7.095	394.96	0.001
Residual	36	648.01		648.01	

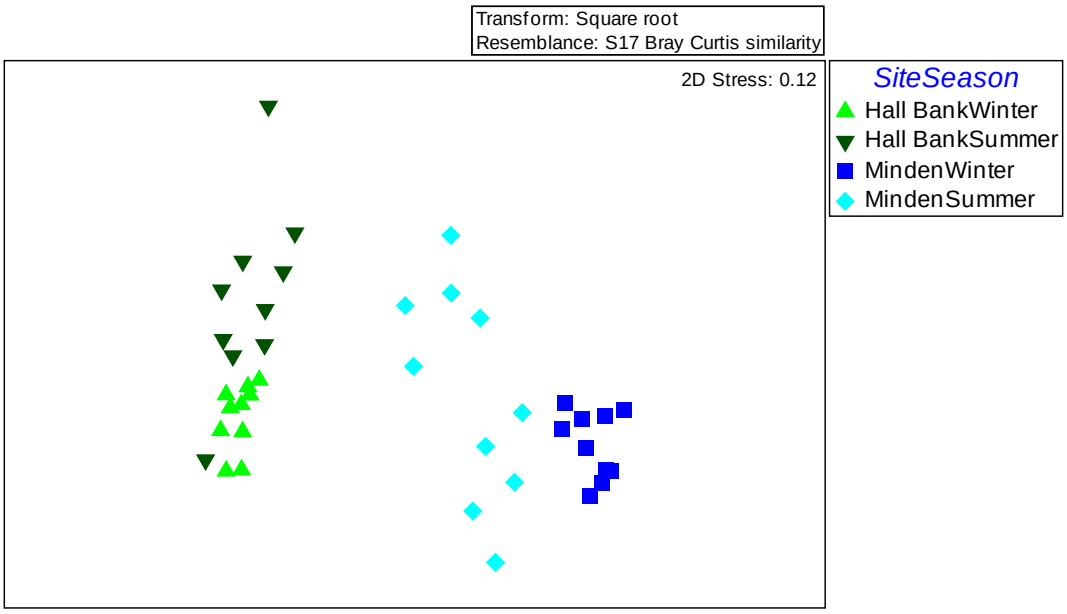

Figure 4.13 nMDS ordination plot, constructed from Bray-Curtis matrices of the percentage frequency of the various dietary items of *C. tenuispinus* in winter and summer in Hall Bank reef and Minden Reef.

SIMPER analysis revealed that the main contributors to *C. tenuispinus* diet in Hall Bank were red turf algae (31.95%), spicules (17.67%) and foraminiferans (10.54%). On the other hand, brown foliose algae (22.37%), red turf algae (15.57%) and red macroalgae (14.92%) mainly contributed to the diet of *C. tenuispinus* in Minden Reef.

Red turf algae and spicules were most abundant in diets of these urchins in winter (red turf algae = 54.83 ± 2.27%, spicules = 16.37 ± 0.87%) (mean ± SE) and summer (red turf algae = 37.93 ± 5.88%, spicules = 17.56 ± 3.37%). Similarly, *C. tenuispinus* feed on red turf algae in Minden Reef in winter (29.28 ± 5.85%). On the other hand, the higher mean frequency of foliose brown algae was observed in diets in winter in Minden Reef. Urchins from Minden Reef seem to feed more on seagrass than urchins at Hall Bank reef (Figure 4.14).

Animal tissues in *C. tenuispinus* diets was comprised of foraminiferans, spicules, poriferans, polychaetes, gastropods, bivalves, bryozoans, arthropods and colonial hydrozoans (Figure 4.14 / 4.15 / 4.16).

Animal components in urchin's guts from Hall Bank reef were dominated by spicules in winter (16.37 ± 0.87%), and in summer by spicules and (17.56 ± 3.37%) foraminiferans (16.78 ± 4.04%). By contrast, the animal component of urchin diets from Minden Reef was dominated by colonial hydrozoans in winter (3.69 ± 0.78 %) (Figure 4.14).

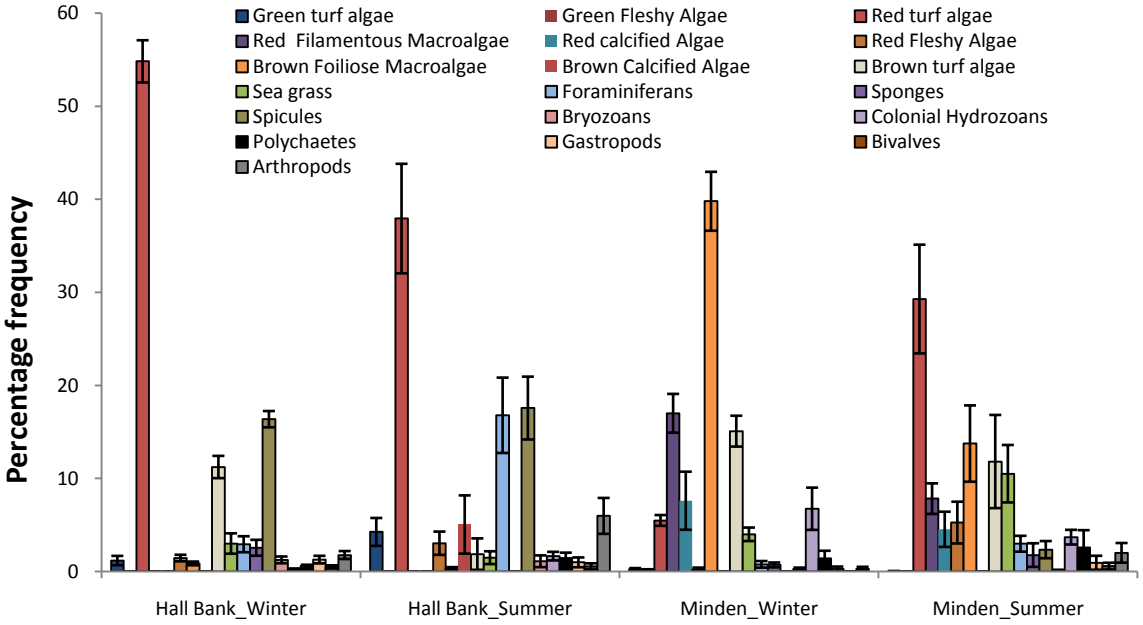

Figure 4.14 Variation of percentage frequency (mean ± SE) of diet components in *C. tenuispinus* diet in Hall Bank Reef and Minden Reef in winter and summer (n = 10 per season/site).

The diversity of dietary items was higher in Minden Reef (summer = 24, winter = 21) compared to Hall Bank reef. Number of macroalgae species (*Codium* sp., *Jania* sp., *Laurencia* sp., *Sargassum* sp., kelp etc.) was abundant in diets of urchins in Minden Reef in winter (Figure 4.18). All the animal taxonomic groups were present in *C. tenuispinus* diets in both sites (Table 4.4).

Table 4.4 Frequency of dietary components in *C. tenuispinus* gut from Hall Bank Reef and Minden Reef in winter 2016 and summer 2017 (mean ± SE), (n = 40).

	Hall Bank		Minden Reef	
Species	Winter	Summer	Winter	Summer
Enteromorpha sp.	1.18±0.50		0.22±0.14	0.03±0.02
Codium sp.			0.12±0.11	
Cladophora sp.		4.25±1.50		
Polysiphonia	48.61±3.08	37.93±5.88	2.66±0.64	22.69±5.56
Herposiphonia sp.	6.22±2.40		1.16±0.33	1.19±0.50
Ceramium sp.			1.67±0.47	
Antithamnion sp.				4.5±1.35

Hypnea sp.			16.17±2.19	
Laurencia sp.			0.22±0.19	5.26±2.24
Jania sp.			7.61±3.12	4.53±1.89
Pterosiphonia sp.				0.43±0.42
Other red algae			0.84±0.35	7.83±1.64
Red fleshy algae	1.45±0.35	3.04±1.24		
Sargassum sp.			18.26±2.40	5.74±1.80
Kelp			5.70±0.59	0.94±0.58
Padina sp.		5.05±3.13		
Sphacelaria sp 1		1.70±1.70		2.71±1.40
Sphacelaria sp 2			15.08±1.66	9.11±4.40
Dasya sp.				0.48±0.48
Other brown algae	0.87±	0.30±	15.83±1.59	7.07±2.08
Seagrass	3.01±1.10	1.48±0.68	4±0.72	10.51±3.09
Feldmannia mitchelliae	11.23±1.19	0.17±0.16		
Foraminiferans	2.93±0.85	16.79±4.04	0.77±0.35	2.98±0.85
Sponges	2.55±0.85		0.71±0.26	1.77±1.27
Spicules	16.37±0.87	17.57±3.37		2.35±0.92
Bryozoans	1.25±0.36	1.10±0.63	0.28±0.13	0.09±0.09
Colonial hydroids	0.23±0.09	1.65±0.46	6.74±2.27	3.69±0.79
Polychaetes	0.57±0.15	1.45±0.57	1.42±0.80	2.59±1.84
Gastropods	1.28±0.40	0.98±0.53	0.26±0.26	0.94±0.75
Bivalves	0.5±0.16	0.55±0.52		0.57±0.37
Arthropods	1.77±0.42	5.98±0.33	0.28±0.21	2.01±1.05
No of prey items	16	16	21	24

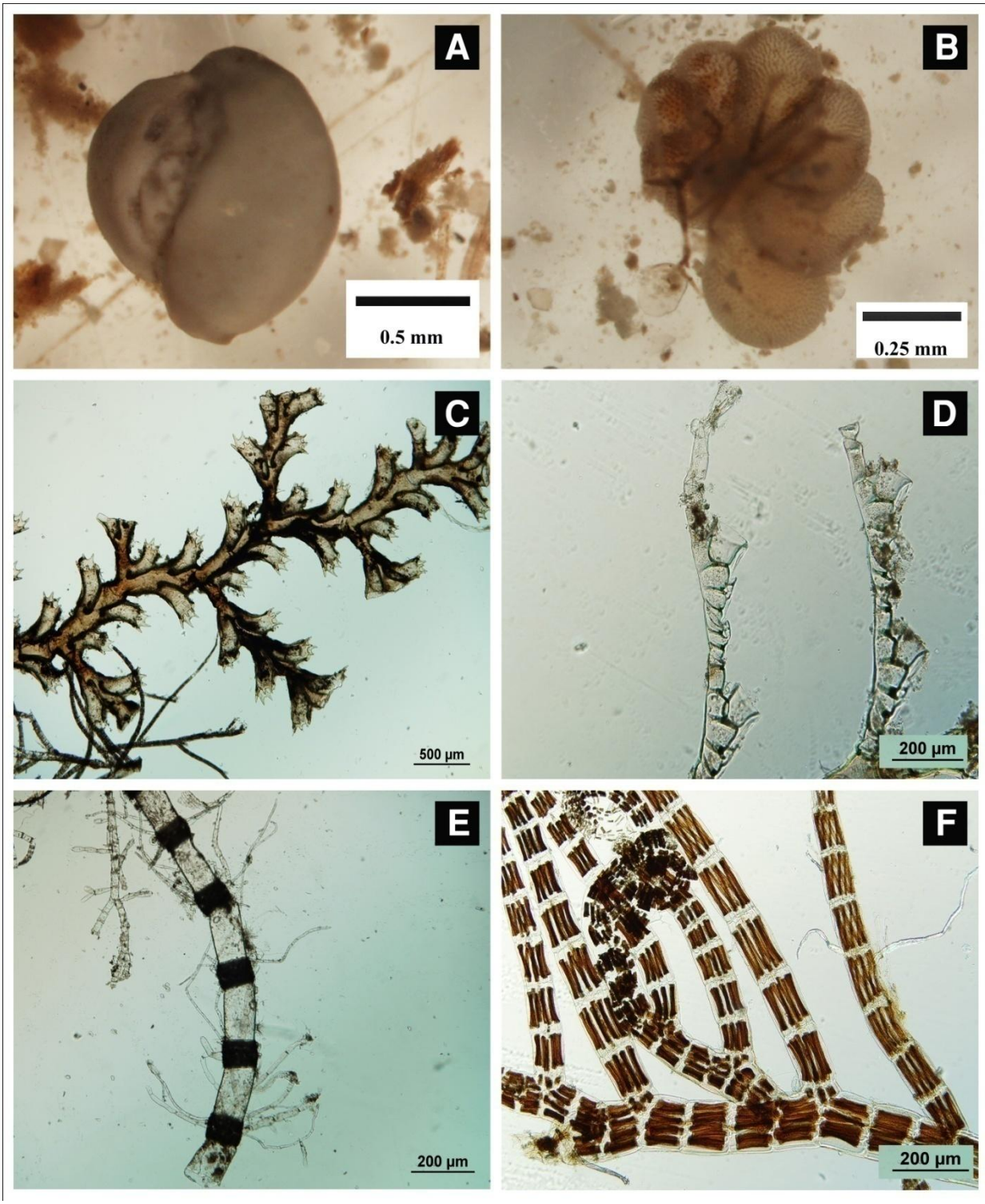

Figure 4.16 Images of different animal and algae species in C. *tenuispinus* diet in Minden Reef (A - B: foraminiferans, C - D: colonial hydrozoans, E - *Ceramium* sp., F: - *Herposiphonia* sp.).

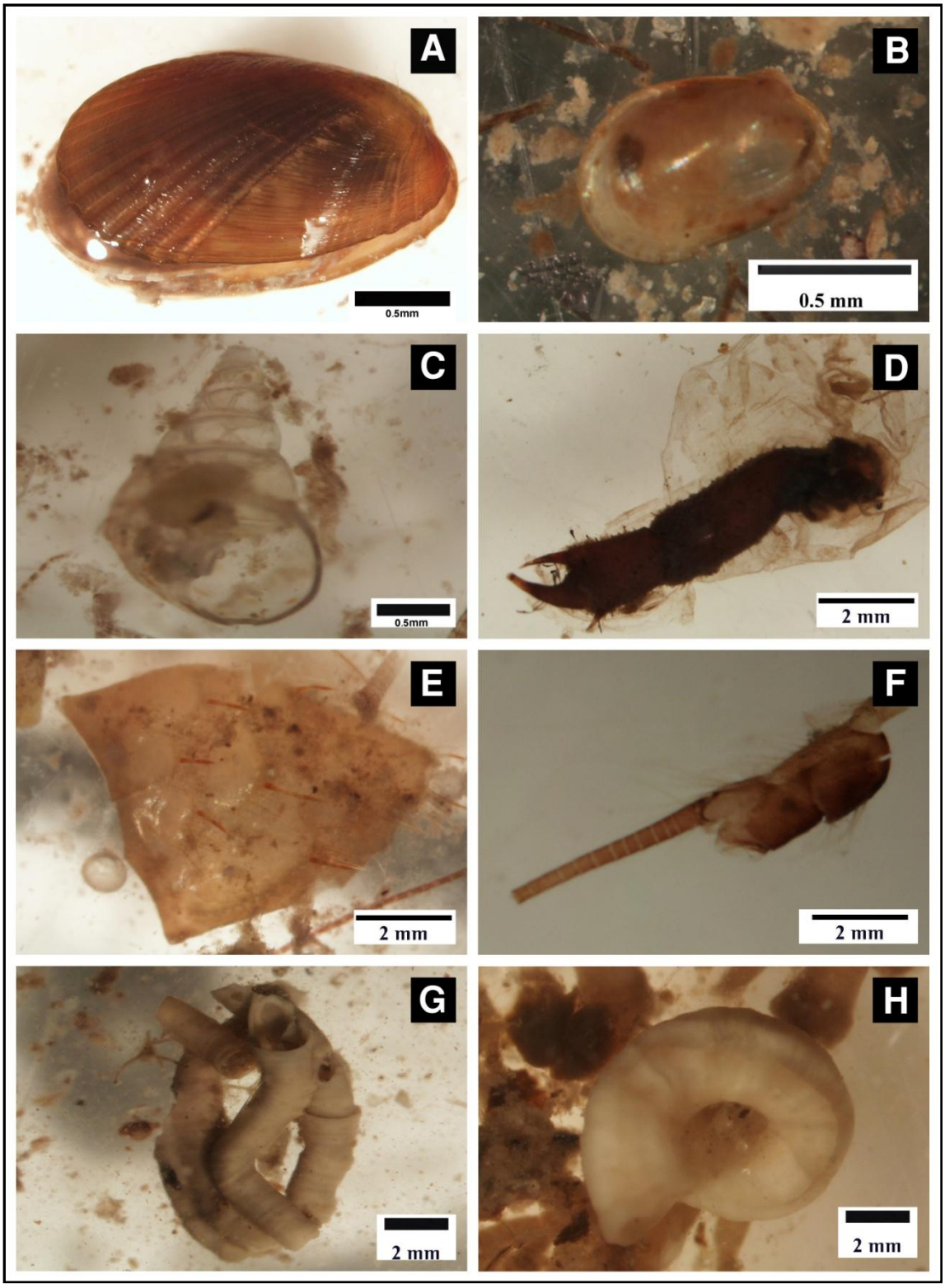

Figure 4.17 Images of different animal species present in the diet of *C. tenuispinus* in Minden Reef (A - B: bivalves, C - gastropod, D-F: arthropod appendages, G - H: polychaetes).

Figure 4.18 Images of different algae species present in Minden Reef (A: *Sphacelaria* sp.1, B: *Sphacelaria* sp.2, C:*Pterosiphonia* sp., D:*Antithamnion* sp., E: *Jania* sp., F: *Hypnea* sp., G: *Sargassum* sp., H: seagrass).

4.3.2.2 Comparison of *C. tenuispinus* feeding rates in Hall Bank and Minden Reefs

Food ingestion rates of *C. tenuispinus* vary between winter and summer (2-way ANOVA, $F_{(3,40)} = 129.143$, $p < 0.001$). Higher food ingestion rates were recorded in summer in Hall Bank reef (5.14 ± 0.34 gut dry weight individual^{-1}day^{-1}) (mean ± SE) and Minden Reef (6.18 ± 0.45gut dry weight individual^{-1}day^{-1}) (Table 4.5). Higher food ingestion rates were recorded in Minden Reef compared to Hall Bank reef ($F_{(1,40)} = 6.595$,$p = 0.015$).

Table 4.5 Food ingestion rate, grazing rate and CaCO3ingestion rate (mean ± SE) *of C. tenuispinus* from Hall Bank reef and Minden Reef (n = 40).

	Halls Bank reef		Minden Reef	
	Winter	Summer	Winter	Summer
Food ingestion rate (gut dry weight individual^{-1} day^{-1})	1.87±0.12	5.14±0.34	2.42± 0.20	6.18±0.45
Grazing rate (gut dry weight individual^{-1} day^{-1})	0.15±0.01	0.45±0.03	0.76±0.05	1.56±0.10
$CaCO_3$ingestion rate (g $CaCO_3$ individual^{-1}day^{-1})	1.65±0.11	4.55±0.30	1.47±0.19	4.03±0.37

Significant differences in grazing rates were also observed between Hall Bank reef and Minden Reef ($F_{(1,40)} = 209.26$, $p < 0.001$), and between seasons ($F_{(1,40)} = 85.24$, $p < 0.001$) (Table 4.4). The interaction between site and season was also significant ($F_{(3,40)} = 17.893$, $p < 0.001$). Yet, $CaCO_3$ ingestion rates were different only with respect to the season ($F_{(1,40)} = 129.14$, $p < 0.001$).

4.3.3 Determining the trophic position of *C. tenuispinus*

Stable isotope values for $\delta^{15}N$ for algae ranged from 3.07 ± 0.35‰ to 4.25 ± 0.04‰. $\delta^{13}C$ for algae ranged from 14.36 ± 0.8‰ to 28 ± 1.54‰. *Feldmannia* sp. had the highest $\delta^{15}N$ among other algae. The highest $\delta^{13}C$ was recorded for *Polysiphonia* sp. 28 ± 1.54‰.

Individual $\delta^{15}N$ of sea urchin muscles ranged 8.38-9.40‰ in Hall Bank reef and 9.15-10.38‰ in Minden Reef. The mean $\delta^{15}N$ for urchins from Hall Bank reef in winter was 8.83 ± 0.53‰ and summer were 8.75 ± 0.37‰. Mean $\delta^{15}N$ for urchins from Minden Reef in winter was 9.80 ± 0.43‰ and summer was 9.79 ± 0.42‰.

Calculated values for trophic position for *C. tenuispinus* in Hall Bank reef were 4.4 and 4.1 in winter and summer respectively. Urchins from Minden Reef had trophic level values of 4.7 and 5.1 in winter and summer respectively.

$\delta^{15}N$ ($\Delta_{\text{urchin – macro and microalgae}}$) was significantly higher for urchins from Minden Reef than that of urchins from Hall Bank reef ($F_{(1,20)} = 25.494$, $p < 0.001$). No seasonal

differences were observed for trophic position for both sites ($F_{(1,20)} = 0.049$, $p = 0.827$). The interaction between site and season also significant ($F_{(3,20)} = 6.441$, $p = 0.023$).

4.4 Discussion

Sea urchins are important grazers (Lawrence 1975), and known for altering their feeding habits in response to food availability and nutritional value of the food (Cabanillas-Terán et al. 2016; Glynn et al. 1979; Livore and Connell 2012a). Their capability of shifting the ecological state of habitats is well known and has been studied in many geographical locations globally (Carpenter 1990; Lawrence 2013; Scheibling 1986). This study confirms that *Centrostephanus tenuispinus* is also capable of altering their feeding habits in response to food availability, depending on temporal and spatial factors. Further, they are active grazers as suggested by Vanderklift et al. (2006).

The diet composition of *C. tenuispinus* reflects the low food availability in Hall Bank and high food availability in Minden Reef (Chapter 2). Temporal variations (seasonal) in availability of food sources also influenced the variations observed in *C. tenuispinus* diets. This is reflected by differences in dry gut weights, urchin test sizes, and diversity and abundance of dietary items in the two study sites. The higher dry gut weights in Minden Reef urchins reflect the high abundance of food in this habitat. Although seasonal differences are not significant, slightly lower gut dry weight at Minden Reef in summer indicated the low abundance of dietary items in summer. In addition, higher frequency of macroalgae in diets and large test sizes are the main contributing factors for high gut weights at Minden Reef in winter (Chapter 2). Higher repletion index at Minden Reef in winter also confirms higher food availability compared to Hall Bank Reef and Minden Reef in summer. The larger size of individuals is the main indication of high food availability in habitat and urchins inhabiting kelp forests are well known to have large sizes in contrast to urchins inhabiting barrens in many geographical regions (Byrne et al. 1998; Ling and Johnson 2009).

High $CaCO_3$ and low organic content reflects the active grazing of *C. tenuispinus* year round, in Hall Bank. Slightly higher $CaCO_3$ percentages at Minden Reef in summer indicate less intense grazing due to high macroalgae availability in winter. Vanderklift et al. (2006) recorded similar observations on diets of *C. tenuispinus* at nearby Mewstone Reef, Stragglers Rocks and Carnac Island. Further, they recorded percentage frequency of calcareous and rock/sand fragments in *C. tenuispinus* was 9.5 ± 2.7%, which was higher than that of other co-occurring urchins *Heliocidaris erythrogramma* (0.8 ± 0.2%) and *Phyllacanthus irregularis* (3.7 ±1.3%) (mean ± SE).

Calcareous and rock / sand fragments in urchin diets were not included in frequency calculations since $CaCO_3$ and siliceous components were quantified separately with chemical methods (Carreiro-Silva and McClanahan 2001). Only organic and siliceous components were included in the percentage frequency calculation. The frequency of these fragments was higher than that of any other organic diet component. Differences in $CaCO_3$ content in urchin's guts at the two study sites reflect the differences in grazing intensity. Sea water temperature is also known to impact the grazing rates of urchins; the higher grazing rate of *C. tenuispinus* recorded in summer is mainly in response to higher sea water temperatures.

In contrast to the study of Vanderklift et al. (2006) on three other reefs in the region, diets of urchins in Hall Bank were mainly composed of turf algae, which could also be attributed to the high abundance of turf algae in the habitat. Although Minden Reef urchins had a higher percentage of macroalgae in their diets compared to Hall Bank reef, the frequency was lower than that of the three reefs studied by Vanderklift et al. (2006). Further, significant differences in abundance of macro algae in summer and winter in Minden Reef are directly influenced by the differences in seasonal abundance of dietary items in habitat (Chapter 2). *Centrostephanus tenuispinus* in Minden Reef tend to feed on more brown foliose algae (*Sargassum* sp.) in winter than summer, which could be related to the high abundance of *Sargassum* sp. in the habitat (Chapter 2). *Sargassum* sp. growth cycles are known to be initiated in autumn, and they reproduce in late August–early September in temperate reefs (Kendrick and Walker 1994). They decline rapidly after late summer which should be the main reason for low macroalgae in urchin's guts in summer of Minden Reef. The urchins may graze on attached turf algae due to lack of macroalgae in summer. It is well known that food availability impacts the foraging behaviour of organisms (Livore and Connell 2012b). On the other hand, seagrass is highly abundant in Minden Reef. Although sea urchin species such as *Tripneustes gratilla* are well known for their preference for seagrass (Klumpp et al. 1993), *C. tenuispinus* scarcely feeds on seagrass.

Higher frequency of *Polysiphonia* sp. in Hall Bank reef was the main the reason for its high abundance in urchin diets (Chapter 2). *Tripneustes gratilla* is known to prefer *Syringodium isoetifolium* due to its high availability and palatability (Lyimo et al. 2011; Vaïtilingon et al. 2003). In addition, *Feldmannia* sp., blue green algae, *Ceramium* sp. and *Cladophora* sp. were present in Hall Bank reef, and were present in *C. tenuispinus* guts. *Tripneustes gratilla* is a generalist herbivore that feeds on a broad range of algae and seagrass; it is known to prefer some red and green algae over others.

However, no discrimination has been observed among brown algae species (Seymour et al. 2013). The lower percentage of macroalgae and seagrass reflects the low availability of these in the habitat itself. The macroalgae recorded in their diets were drifting algae which could not be identified to species level. Urchins are known to switch their feeding habits from grazing to trapping drifting algae; *C. tenuispinus* may catch drifting algae which originates from nearby macroalgae-dominated reefs. Since Hall Bank reef is surrounded by seagrass meadows, seagrass present in the gut could have originated from drifting seagrass fragments trapped by these urchins. Drifting algae is known to provide food for organisms in low productive subtidal habitats in many geographical regions. Drifting kelp exported from highly productive macroalgae-dominated reefs are an importantsource of energy for subtidal urchins (Britton-Simmons et al. 2009; Kelly et al. 2012). Although *Padina* sp. is visually absent from Hall Bank reef (Chapter 2), the presence of early growth stages in *C. tenuispinus* gut reveals that they control the growth of algae through grazing.

With respect to the main algal divisions, the most abundant dietary category was red algae in Hall Bank reef, irrespective of season. In Minden Reef, brown algae were highly abundant in the diet of *C. tenuispinus* in summer. Similarly, a study of *C. tenuispinus* diets in Mewstone Reef, Stragglers Rocks and Carnac Island showed that they feed mainly feed on red algae (40.2 ± 5.3%) and brown algae (36.6 ± 7.9%) (Vanderklift et al. 2006). Seagrass seems to be least preferred by these urchins despite high abundance in urchin's guts from Minden Reef in both winter and summer.

The contribution of animal tissues to the diet of *C. tenuispinus* at Hall Bank was higher than at Mewstone Reef, Stragglers Rocks and Carnac Island (10.3 ± 3.1%) (Vanderklift et al. 2006). *C. tenuispinus* in Hall Bank reef is known to feed on spicules, sponges, foraminiferans, bryozoans, colonial hydroids, arthropods, gastropods and bivalves. The high percentage of spicules recorded in the urchin diet could be due to both incidental ingesion while grazing, and active grazing on sponges, bryozoans or corals. Foraminiferans could be grazed with sediment or epiphytes of drifting seagrass or macroalgae. Vanderklift et al. (2006) has also recorded animal tissues in diets of *C. tenuispinus* which could be derived from ascidians and sponges. Other diadematoid species such as *Diadema* sp. are known to feed on sponges, hydroids, bryozoans, nematodes, rotifers, gastropods, bivalves and copepods (Hernández et al. 2006; Rodríguez-Barreras et al. 2015a). Higher percentages of animal material in urchin guts at Hall Bank are mainly the result of unintentional ingestion during active grazing. Although there are fewer foraminiferans and spicules in urchin's guts from Minden Reef

in summer, other animal components ingested are more abundant, which could be mainly due to shifting in their feeding habits to active grazing on encrusted benthic species. On the other hand, spring and summer are the seasons in which most species spawn. Thus, juveniles and recruits are highly abundant in summer and autumn. A high abundance of epiphytic communities also has been recorded in many temperate regions in summer and autumn High percentage siliceous components in urchins diets of Minden Reef, could be due to indirect ingestion of sand particles.

Hall Bank reef is mainly dominated by *C. tenuispinus*. No other urchin species were observed in this site except one individual of *Phyllacanthus irregularis* in June 2015. In Minden Reef, *C. tenuispinus* coexists with *Heliocidaris erythrogramma* and *Phyllacanthus irregularis*. These three urchins are also known to co-occur in Mewstone Reef, Stragglers Rocks and Carnac Island. *Heliocidaris erythrogramma* is well known for its herbivory and feeding on drifting algae. On the other hand, *C. tenuispinus* and *Phyllacanthus irregularis* are known for their omnivory in these reefs (Vanderklift et al. 2006). Co-occurring urchins within the same habitat are known to have diverse food preferences, feeding mechanisms or feeding behaviours (Contreras and Castilla 1987). *Centrostephanus tenuispinus* in Mewstone Reef, Stragglers Rocks and Carnac Island is also known to have a similar trophic level (4.5 ± 0.2‰) (mean ± SD). The co-existing urchins *Heliocidaris erythrogramma* and *Phyllacanthus irregularis* are known to have trophic values of 2.7 ± 0.4‰ and 4.6 ± 0.6‰ respectively. Niche separation has been observed in co-occurring urchins *Loxechinus albus* and *Tetrapygus niger* in Central Chile, *Arbasia lixula* and *Paracentrotus lividus* in the Mediterranean, and *Ctenocidaris gigantea, C. spinosa, Notocidaris mortenseni, Sterechinus antarcticus* and *S. neumayeri* in Antarctic waters (Contreras and Castilla 1987; Jacob et al. 2003). Wangensteen et al. (2011) have documented differences in $\delta^{15}N$ of coexisting Mediterranean urchins *Arbasia lixula* (8.2 ± 0.5‰) *Paracentrotus lividus* (5.9 ± 0.4‰), which indicates the niche separation in the habitat. Higher $\delta^{15}N$ could not be explained by algae $\delta^{15}N$ in *C. tenuispinus* diets. Higher trophic level values indicated their omnivorous nature. Although urchin diets in Minden Reef had low percentage of animal components compared to Hall Bank reef, higher $\delta^{15}N$ could be due to high assimilation of the animal components and high nutritive values of animal tissues in Minden Reef. Spatio-temporal variation in the trophic level of *Diadema antillarum* (2.35 ± 0.11 - 3.34 ± 0.17) was also recorded by (Rodríguez-Barreras et al. 2015a). These values can only be explained by high ingesion of invertebrates. *Centrostephanus rodgersii* in eastern Australia is also known to have an omnivorous foraging habit (Andrew 1993).

Sea water temperature is known to impact the feeding rates of urchins (Chapter 3). Further, the reproductive cycle of *C. tenuispinus* is was influenced by sea water temperature and day length (Chapter 3). The gametogenic cycle of *C. tenuispinus* is initiated in March with the onset of autumn (Chapter 3). Further, in summer gonads are in the recovery stage, gathering energy and nutrients for the next cycle. A higher proportion of animal components in the diet in autumn could also influence the initiation of the gametogenic cycle. Significant differences in diet composition in March to the rest of the year (except June) could coincide with initiation of the gametogenic cycle. These urchins spawn in July-August (winter), and in spring and early summer, gonads are mostly in the recovering stage. Higher amounts of microalgae in *C. tenuispinus* diet in spring could be due to two reasons: greater feeding after spawning and high abundance of turf algae in the habitat. Further, most of the animal components were high during the initiation of the gametogenic cycle. High protein content in the diet is known to produce high quality gonads (Jong-Westman et al. 1995). The urchins feed less during the spawning months. It is well known that urchins tend to feed on more nutritious food items during the reproductive periods. The impact of food supply (quantity) and nutritional value of food (quality) on gonad condition have been well studied in both field and laboratory conditions (Keats et al. 1984; Meidel and Scheibling 1999; Minor and Scheibling 1997; Phillips et al. 2010; Shpigel et al. 2005). Urchins inhabiting kelp beds are known to have higher gonad indices and have higher organic content compared to those inhabiting barrens (Meidel and Scheibling 1998).

This study revealed that *C. tenuispinus* is an omnivore that tends to actively graze on reef substrate. Temporal variation in its diet composition is directly influenced by the extent of the food supply in the habitat, in response to environmental factors. Further, the capability of *C. tenuispinus*, shifting its feeding behaviour in response to food availability, confirms its potential for surviving in new habitats.

Chapter 5 - Role of *Centrostephanus tenuispinus* as a bio-eroder in Hall Bank Reef

5.1 Introduction

5.1.1 Role of Bio-erosion in reef carbonate budgets

Sea urchins are the only echinoderms capable of significant bio-erosion in benthic ecosystems (Glynn and Manzello 2015). Since their intense grazing impacts their habitat immensely through changing the biodiversity and altering the physical habitats tructure, they act as habitat modifying organisms in many shallow reefs and intertidal habitats (Davidson and Grupe 2015).

Among other shallow benthic ecosystems, sea urchins play a key role in coral reefs, impacting reef diversity and structure. The three-dimensional vertical carbonate structure of corals is continuously subjected to accretion and erosion processes. Reef forming stony corals accumulate calcium carbonate in their skeletons, forming the reef structure; this structure is further strengthened by encrusting calcareous algae. On the other hand, both biotic and abiotic processes influence reef erosion. It is well-known that many invertebrates and fish species inhabiting the reef are capable of removing hard reef material through their foraging mechanisms, causing bio-erosion (Appana and Vuki 2006; Dumont et al. 2013; Glynn and Manzello 2015; Hutchings 1986). The balance between reef accretion and erosion is critical in maintaining the health of reefs (Alvarado et al. 2016; Glynn and Manzello 2015; Tribollet and Golubic 2011).

In general, many bio-eroders that excavate into reef structure weaken the reef skeletal structure and contribute sediment to the reef environment. Sediment produced by bio-eroders is known to accumulate in the reef or can be transported to a different location (Perry et al. 2015). In extreme bio-erosion events, the collapse of reef frame work is possible, with an accumulation of sediment in the reef eventually burying reef frame-building species and reducing topographic complexity (Glynn et al. 2017; Glynn and Manzello 2015). The extent of sediment accumulation is very critical for the health of the reef.

Glynn and Manzello (2015) suggested that moderate bio-erosion could contribute to the accumulation of sedimentary substrate for reef-associated species, increasing topographic complexity to host many species. This is known to increase biodiversity, alter reef morphology and promote regeneration and rejuvenation of senescent reef building organisms. It is well documented that bio-erosion is vital for reef development and maturation (Glynn and Manzello 2015).

As previous studies suggest, the level of bio-erosion is dependent on many factors; morphological structure and skeletal density of corals, water quality, diversity of bio-eroder community, nature of the bio-eroders (macro or micro) and size and population structure of bio-eroders (Brown-Saracino et al. 2007; Bruggemann et al. 1996; Highsmith 1981; Holmes et al. 2000; Reaka-Kudla et al. 1996). Coral structures in reefs are known to be more susceptible to bio-erosion than hard limestone pavement structures (Bruggemann et al. 1996; Reaka-Kudla et al. 1996).

5.1.2 Diversity of bio-eroders

Bio-eroders are represented by many taxonomic groups including bacteria, fungi, algae and other invertebrates such as mollusc, crustaceans, sponges and echinoderms (Glynn and Manzello 2015). These organisms use different mechanisms to break down the reef material (Bruggemann et al. 1996). Mechanical, chemical or combinations of both methods have been used by the diverse array of bio-eroders. On the other hand, the microhabitat of the bio-eroding agent is also known to determine the extent of bio-erosion.

Golubic et al. (1975) categorised bio-eroders into epiliths (species live on reef surface), chasmoliths (species inhabit holes and cracks) and endoliths (species live inside the skeleton). Many bio-eroders are capable of occupying one or more microhabitats during different stages of life. Boring or excavating eroders have been reported to remove relatively large amounts of calcareous material and weakening the reef structure. Many epilithic bio-eroders are grazers that scrape on substrate algae and the erosion caused by them is associated with feeding. Grazing by chitons, gastropods, sea urchins and parrotfish contributes large amounts of sediment to the reef habitat (Bak 1994; Bellwood 1996; Peyrot-Clausade et al. 2000). Irrespective of internal or external inhabitancy, the borer abundance also impacts the extent of the erosion. Although reef destruction by endolithic bio-eroders is less conspicuous and rarely documented, reef destruction by epilithic species such as sea urchins has been documented in many reefs (Bak 1994; Bronstein and Loya 2014; Carreiro-Silva and McClanahan 2001; Glynn 1988).

5.1.3 Role of sea urchins as bio-eroders

Among other bio-eroders, sea urchins play a vital role in many marine benthic habitats (Andrew and Underwood 1989b; Bluhm et al. 2009; Mamelona and Pelletier 2005; Mokady et al. 1996; Scheibling 1986; Tuya et al. 2004). Sea urchins in high densities are capable of converting rich algae-dominated reefs into red coralline algae-

dominated barrens (Abraham 2007; Ling et al. 2010). Moderate densities of sea urchins are known to remove competitive algae, reducing coral mortality due to algal overgrowth and allowing corals to increase in abundance (Bluhm et al. 2009; Coyer et al. 1993; Hernández et al. 2008). Although urchins are widely recognised to be keystone grazers and their grazing habits have been extensively studied, their role as bio-eroders has been poorly studied in many temperate reefs. Sea urchins being one of the major causative agents of bio-erosion (Bak 1994; Glynn 1988; Mokady et al. 1996) and dominant benthic grazers in many temperate coastal areas (Scheibling 1986; Tuya et al. 2004) have an immense impact on its habitats itself.

Sea urchins are capable of weakening the carbonate reef through two ways; spine abrasion, and the feeding process via the Aristotle's lantern (Klinger and Lawrence 1985). The Aristotle's lantern is highly specialised and is composed of five calcified teeth. These teeth are composed of plates and needles embedded in a magnesium-enriched polycrystalline matrix. The teeth act as scraping plates and are very efficient in eroding the substrate (Killian et al. 2011; Ma et al. 2009; Ma et al. 2008). Some species of urchins excavate into the reef and make burrows during feeding, and rarely leave the burrow (McClanahan and Kurtis 1991). Erosion caused by spines enlarges the burrow. Thus, high densities of these urchins can weaken the reef structure and impact reef stability (Asgaard and Bromley 2008; Griffin et al. 2003). Sea urchin species of the genera *Echinometra, Echinostrephus*, *Diadema,* and *Eucidaris,* are known to impact reefs by excavating (Bak 1994; Dumont et al. 2013; McClanahan and Kurtis 1991; Reaka-Kudla et al. 1996). *Echinometra lucunter* in the Caribbean Sea and the Atlantic Ocean has been reported to produce two types of boring; tube-like elongated grooves and cup-shaped burrows (Asgaard and Bromley 2008). *Echinometra mathaei*, *Echinometra viridis*, *Eucidaris galapagensis*, *Diadema antillarum*, *Diadema mexicanum*, *Diadema savignyi* and *Echinothrix diadema* are well known for their impact on reefs in the Caribbean Sea, and the Atlantic and west Indian oceans (Bak 1990; Bak 1994; Glynn and Manzello 2015; Glynn et al. 1979).

Sea urchins contribute to the balance between reef constructive and reef destructive processes through bio-erosion. The extent of bio-erosion is critical for reef health. When bio-erosion exceeds reef accretion levels, reef health is in jeopardy. In tropical reefs bio-erosion of 40 kg $CaCO_3\,m^{-2}\,a^{-1}$ has been recorded, which exceeds reef accretion rates (0.3 – 12 kg $CaCO_3\,m^{-2}\,a^{-1}$) (Glynn 1988). The degree of sea urchin bio-erosion depends on many factors. Among those, species, density of the urchin population, size of urchins, feeding ecology and the nature of the habitats were

commonly monitored factors (Bak 1994). The density of the urchin population can cause an impact on reef health to a varying degree from pristine conditions to degraded conditions. According to Bak (1994) urchin bio-erosion can equal or exceed reef carbonate production. The highest rates of average bio-erosion by sea urchins havebeen reported on reef slopes (0.9 kg $CaCO_3$ m^{-2} a^{-1}), then on reef flats (0.5 kg $CaCO_3$ m^{-2} a^{-1}) (Mokady et al. 1996). *Diadema antillarum* has been reported to cause erosion of 4.6 kg CaCO3 $m^{-2}a^{-1}$ on patch reefs of St Croix, USA (9 individual m^{-2}), and 5.3 kg CaCO3 m^{-2} a^{-1} on fringing reefs in Barbados (23 individual m^{-2}). Bio-erosion on exposed reefs was significantly higher than on sheltered reefs (Bronstein and Loya 2014). Further, Carreiro-Silva and McClanahan (2001) observed that despite the similar body sizes bio-erosion could vary due to the different feeding behaviours (species-specific feeding strategies). Bio-erosion rates of *Echinometra mathaei* in Ningaloo Reef has been recorded 0.98 - 4.55 kg $CaCO_3$ m^{-2} a^{-1} in lower limits of tropics 21-23 ° S (Langdon 2012).

5.1.4 Impact of climate change on bio-erosion

The majority of bio-eroders are known to attack dead coral skeletons (Carreiro-Silva and McClanahan 2012; Catarino et al. 2012; Glynn 1988; Glynn and Manzello 2015). Conditions leading to coral mortality increase the activity of bio-eroders: coral mortality caused byseawater temperature changes, terrestrial run off leading to eutrophication, tidal exposure, predation and disease out breaks were recorded with previous studies (Burke et al. 2011; Glynn 1988; Hallock 1988; Holmes et al. 2000; Hughes and Connell 1999; Hughes 1994). Enhanced nutrient levels have increased the epilithic cover in reef areas, leading to changes in grazing pressure and micro-borer communities (Chazottes et al. 2002). Similarly, macro-bio-erosion was positively correlated with chlorophyll *a* levels in sea water (Carreiro-Silva and McClanahan 2012). Increased populations of boring sponges have been recorded in eutrophic conditions on the west coast of Barbados (Holmes 2000).

Elevated carbon dioxide levels leading to ocean acidification is known to impact coral growth and proliferation (Doropoulos et al. 2012; Silbiger et al. 2014). A decline in aragonite saturation state has been observed to reduce coral calcification (Manzello et al. 2014). Acidic conditions can weaken reef structures, favouring the proliferation of bio-eroder populations (Glynn and Manzello 2015; Wisshak et al. 2012). Over-fishing of many predatory species has also enhanced the proliferation of many bio-eroding species, increasing the extent of bio-erosion (Brown-Saracino et al. 2007; O'Leary et al. 2012; Sheppard-Brennand et al. 2016).

Coral reef growth has been the focus of many biological studies for the last few decades. Reef degradation by anthropogenic influences has gained attention more than ever in the history; yet, reef destruction by bio-erosion has received little attention (Tribollet et al. 2002). Thus, quantification of this impact is essential to understand and manage many marine habitats. Results from previous studies suggest the need for species-specific studies on bio-erosion in unique sea urchin dominated habitats. Most studies focused on bio-erosion have been centred on tropical reefs, mainly Caribbean and Indo-Pacific regions, while bio-erosion of reefs at higher latitudes is poorly studied.

5.1.5 *Centrostephanus tenuispinus* as bio-eroders at Hall Bank Reef

Diadematoid urchins have been documented for their intense bio-eroding ability in tropical regions. *Diadema* spp. and *Echinothrix* spp. have been the subject of many bio-erosion studies in the tropics and are known to have a high impact on their habitat (Bak 1994; Carreiro-Silva and McClanahan 2001). *Centrostephanus* is a diadematoid sea urchin which is capable of creating barrens (Byrne et al. 1998; Ling and Johnson 2009). Although *Centrostephanus rodgersii* on the east coast of Australia has been studied extensively due to its range extension towards the south and extensive grazing, their impact as bio-eroders has never been quantified. *Centrostephanus tenuispinus* is also known as a grazer that actively feeds on attached algae on the west coast of Australia. Previous studies of their gut contents have revealed the presence of rock and coral fragments, confirming their extensive grazing ability (Vanderklift et al. 2006).The presence of *C. tenuispinus* in high densities (5.0 ± 0.81 m^{-2}) at Hall Bank Reef, Western Australia (32° 2.002´S and 115° 42.957´E) was reported by Thomson and Frisch (2010). Hall Bank Reef is known for an unusual absence of macroalgae and presence of high coral cover (mean coral cover = $52.6 \pm 4.65\%$) for this region. Since actively grazing urchins are known to influence the reef environment by removing a substantial amount of reef substrate, studies of *C. tenuispinus* bio-erosion will reveal the importance of their role in reef structuring processes. This study was mainly focused on quantifying the bio-erosion of *C. tenuispinus* in Hall Bank Reef with respect to urchin size structure and seasonality.

5.2 Methodology

5.2.1 Study Site

This study was conducted at Hall Bank Reef, Western Australia (32° 2.002´S and 115° 42.957´E) (Chapter 2).

5.2.2 Sampling procedure

Bio-erosion rates were calculated based on the percentage of calcium carbonate in urchin gut contents and the gut evacuation rate (Elliott and Persson 1978).

5.2.2.1 Analysis of gut composition

Monthly samples of sea urchins were collected fromDecember 2014 – February 2016 (26 individuals per month). Sea urchin samples were transfered on ice to the laboratary at Murdoch University. Samples were blotted dry with tissue paperbefore processing. Test diameter (vernier calliper, ± 0.1 mm) and wet weight (balance, ± 0.001 g) were measured. Spines were removed, urchins were dissected and gut contents extracted. Wet gut weight was measured (balance, ± 0.001 g). The Aristotle's lantern was removed, and diameter, pyramid height and lantern weight were measured.

Gut content composition (organic, calcium carbonate and other silicious compenents) was determined using the method of Carreiro-Silva and McClanahan (2001). Gut contents were dried to constant weight in a preheated oven at 70ºC for 48 hours and weighed. Subsamples of 1g of the gut contents were weighed on a high precision analytical balance (± 0.0001 g) and were immediately transfered to a combustion chamber for 5 hours at 500 °C to combust the organic matter. Samples were weighed after combustion and digested with 5% HCl. The residual matter was filtered with preweighed filter paper in suction. Finally, filter papers with the residual matter were dried (70 ºC for 8 hours) and weighed. The organic and inorganic proportions of gut contents were calculated using differences between combusted and digested samples.

5.2.2.2 Determination of gut evacuation rate of *C.tenuispinus*

The gut evacuation rate experiment was conducted for winter and summer. Spring and autumn gut evacuation rates were calculated based on winter and summer rates, considering changes in seawater temperature.

Fifty sea urchins were collected for two gut evacuation experiments in August 2015 (winter) and January 2016 (summer). Five urchins were sacrificed at the point of collection (0 hours) and were transferred to ice. Other urchins were transferred to the laboratory and were maintained in a seawater flow-through aquarium (temperature controlled 17 ºC in winter and 22 ºC in summer). These urchins were sacrificed at 4, 8, 12, 16, 24, 36, 48, 72 and 90 hours. Samples were dissected; gut contents were carefully removed from the gut and weighed. Gut contents were dried for 48 h in anoven at 70 °C and weighed. Mean values (n = 5) for dry gut weight were plotted against time. The relationship between dry weight and time was defined by a regression equation:

$C = C_0 e^{-RT}$ ----------1

Where *C* = Gut content weight (g), C_0 = Gut content at time 0, t = time (h), R = Rate of decrease

Mean time (t) for gut evacuation was calculated using Elliot's equation (Elliott 1972):

$\bar{t} = \int_0^{\infty} \frac{C_0 e^{-RT} dt}{C_0} = \frac{1}{R}$ ----------2

Daily calcium carbonate and organic component ingestion were calculated using the following equation:

$F = CR\,24$----------3

Where F = Food ingestion rate (g individual^{-1} day^{-1})

5.2.2.3 Determination of newly eroded calcium carbonate

Since sea urchins tend to ingest $CaCO_3$ in the sediment (previously eroded material / reworked $CaCO_3$), quantifying re-ingested (reworked) $CaCO_3$ is needed for calculation of new erosion. Hence, a control experiment was conducted using 30 sea urchins. These urchins were caged in the adjacent seagrass bed devoid of any coral or calcareous material for grazing to quantify the reworked calcium carbonate (control). Assuming that urchins in this habitat feed only plant and animal components, calcium carbonate percentages from this experiment were used as a correction factor (reworked $CaCO_3$) to calculate newly eroded percentage of calcium carbonate.

Thirty urchins were caged (mesh size 1 X 1cm) in five cages (1m X 1m X 0.75m) (six urchins per cage) in a seagrass bed adjacent to the Hall Bank reef (15m deep). Cages were kept underwater for two months. All sea urchins were sampled after two months and were transferred to the laboratory on ice. Urchins were dissected, and gut contents were removed and weighed. The amount of $CaCO_3$ in the gut was analysed using the method of Carreiro-Silva and McClanahan (2001). The difference in percentage $CaCO_3$ of caged urchins and reef urchins was considered as the percentage of newly eroded calcium carbonate (equation 4).

$\text{Newly eroded } CaCO_3 = \text{Total } CaCO_3 - \text{Reworked } CaCO_3$---------4

5.2.2.4 Estimation of sea urchin density

Ten haphazard belt transects (20 X 1m) were sampled on the reef in four seasons 2015 (autumn, winter, spring and summer). Eight transects were deployed in reef flat depth ranging from 8 - 10m and two trasects deployed in reef slope (11 - 12m). The number of urchins in each transect was counted, and sea urchin density was calculated asnumber of individuals per square meter.

5.2.3 Calculation of bio-erosion rates

The gut content composition was calculated as percentages. Percentage of organic and $CaCO_3$ components and gut evacuation rate were used to calculate bio-erosion rates and food ingestion rates for four seasons (Elliott and Persson 1978):

$$\text{Food ingestion rate} = \text{Daily ingestion rate (g day}^{-1}\text{) X Dry gut content (g)} \quad \text{....5}$$

$$\text{Bio} - \text{erosion rate} = \text{Daily ingesion rate (g day}^{-1}\text{) X Newly eroded } CaCO_3 \text{ weight (g)} \quad \text{....6}$$

5.2.4 Statistical analysis

All sampled sea urchins were categorised into three different size classes depending on test diameter: < 65 mm, 65 – 70 mm and > 70 mm. $CaCO_3$ percentages and bio-erosion rate data were tested for homogeneity and compared with respect to seasonal variation and test size in a two-way ANOVA followed by post hoc tests (Tukey HSD). All statistical tests were carried out in SPSS software (SPSS 24).

5.3 Results

5.3.1. Seasonal variation in *C. tenuispinus* gut composition

Centrostephanus tenuispinus gut content was mainly composed of inorganic components (over 87%). The inorganic component represented 89.68 ± 0.14% (mean ± SD) of the seasonally averaged gut content while organic component was 10.32 ± 2.76% (mean ± SD). Inorganic components for summer 2015 was significantly lower (87.44 ± 3.05%) than other seasons ($P < 0.001$). Winter 2015 inorganic component was significantly higher (91.26 ± 1.77%) than any other season except spring 2015. Summer 2015 had the highest organic component (12.56 ± 3.05%), and winter 2015 had lowest organic component (8.84 ± 1.77%) (Figure 5.1). The proportion of organic and inorganic components ingested varied with the test size (diameter) of the sea urchins ($F_{(2,4)} = 5.226$, $p = 0.006$). Smaller urchins (< 65 mm) ingested the highest percentage of organic components (11.05 ± 2.80%) compared to the two larger urchin size categories. There were no significant differences in the percentage of inorganic components and organic components among size classes of 65 – 70 mm and > 70 mm ($p = 0.830$). Further, autumn 2015, spring 2015 and summer 2016 were similar in inorganic and organic proportion.

5.3.2 Analysis of percentage calcium carbonate in urchin gut content

Calcium carbonate was the main constituent of inorganic component (over 80%) in all seasons (Figure 5.1). The overall means of $CaCO_3$, organic matter and other siliceous components were 86.29 ± 3.23%, 10.32 ± 2.76% and 3.39 ± 1.52% (mean ± SD) respectively. Mean $CaCO_3$ percentage in summer 2015 was significantly lower (83.83 ± 3.73 %) than the other seasons ($F_{(4,364)} = 17.811$, $p < 0.001$). No significant differences in mean $CaCO_3$ percentage were observed between other seasons. Calcium carbonate percentages did not vary between the three test size classes ($F_{(2,364)} = 1.645$, $p = 0.195$).

Seasonal differences were recorded with respect to other siliceous components in the gut ($F_{(4,364)} = 5.202$, $p < 0.001$). Siliceous component ingested in winter 2015 was significantly higher (3.97 ± 1.72%) than other seasons except for summer 2015 (3.61 ± 1.74 %) ($p = 0.570$).

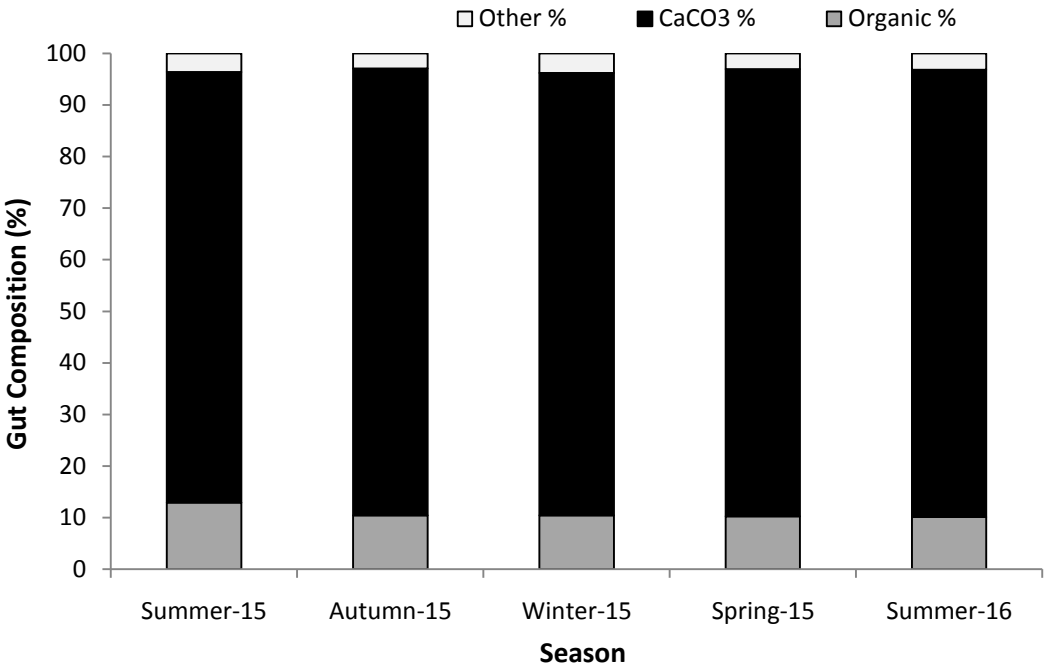

Figure 5.1 Component proportions (mean ± SD) of gut contents in sampled *C. tenuispinus* individuals (n = 364).

The mean seasonal dry gut weight of *C. tenuispinus* was 6.83 ± 1.69g. The highest gut dry weight (7.31 ± 2.02g) was observed in spring 2015 and lowest in summer 2015 (6.48 ± 1.33g). Significant differences in dry gut weight were recorded between three seasons: summer 2015, autumn 2015 and spring 2015 ($F_{(4,364)} = 4.485$, $p = 0.002$) (Table 5.1). Further, considerable variations in dry gut weight were also observed between the three size classes < 65 mm, 65 – 70 mm and > 70 mm ($F_{(2,364)} = 55.177$, $p < 0.001$)(Figure5.2).

Table 5.1 Results of two-way ANOVA of dry gut weight on seasonal and test size as factors (n = 364), (α = 0.05). *denotes significant results.

	Type III corrected Sum of Squares	df	Mean Square	F	Sig.
Corrected model	310.932	14	22.209	10.535	<0.001
Intercept	14684.474	1	14684.474	6965.670	<0.001
Season	37.821	4	9.455	4.485	0.002*
Test size	232.641	2	116.320	55.177	<0.001*
Season*Test size	30.379	8	3.797	1.801	0.076
Error	735.734	349	2.108		
Total	18046.502	364			
Corrected Total	1046.666	363			

The highest amount of ingested food was recorded in urchins of large size class (> 70 mm) irrespective of season. The lowest dry gut weight was observed in size class of < 65 mm (5.81 ± 1.29 g in winter 2015) and highest dry gut weight recorded was for size class > 70 mm (7.74 ± 1.66 g in spring 2015) (Figure 5.2 / Table 5.2).

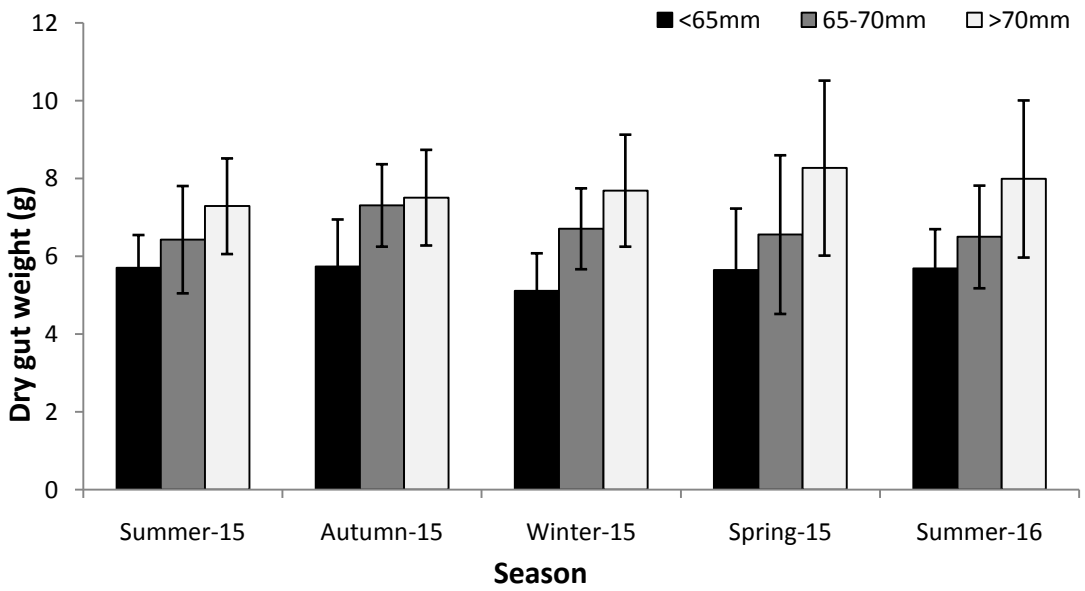

Figure 5.2 Seasonal variation in dry gut weight (g) in three size classes of *C. tenuispinus* (n = 364).

Table 5.2 Mean gut weight, mean CaCO3 weight and mean organic weight of *C. tenuispinus* (mean ± SD, n = 364).

Season	Mean gut weight (g)	Mean $CaCO_3$ weight (g)	Mean organic weight (g)
Summer-2015	6.48±1.33	5.44±1.16	0.81±0.25
Autumn-2015	6.54±1.37	5.67±1.21	0.68±0.20
Winter-2015	6.75±1.63	5.87±1.40	0.59±0.20
Spring-2015	7.31±2.02	6.38±1.77	0.71±0.28
Summer-2016	6.98±1.85	6.06±1.65	0.69±0.18

5.3.3 Determination of newly eroded calcium carbonate

Inorganic and organic components contributed 70.25 ± 4.83% and 29.75 ± 5.24% respectively in the guts of caged urchins. Mean percentage of $CaCO_3$, organic and other inorganic components of the caged urchins were 64.99 ± 5.24%, 29.75 ± 4.83 % and 5.54 ± 2.30% respectively (Figure 5.3). The proportion of the organic component was nearly three times (29.75 ± 4.83%) that of the population studied in the reef habitat (10.32 ± 1.41%) ($p < 0.001$). Mean newly eroded $CaCO_3$ percentage was 21.30 ± 3.23 %.

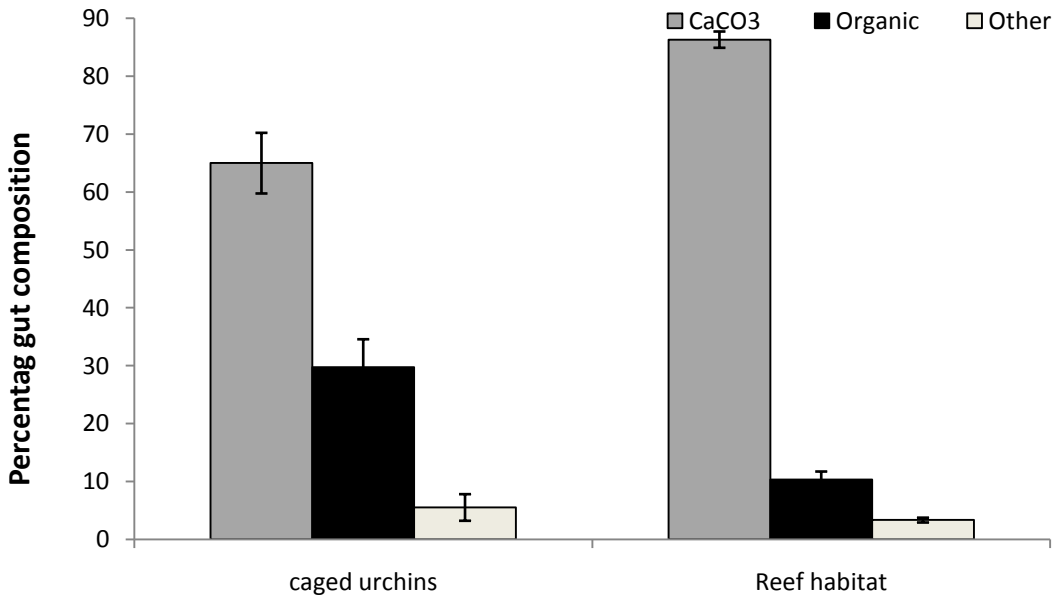

Figure 5.3 Composition of *C. tenuispinus* mean dry gut components in sampled population (n = 364) and caged population in Hall Bank reef (n = 30).

5.3.4 Analysis of gut evacuation rate

Initial dry gut weight in summer and winter was 7.125 ± 0.97 g and 5.198 ± 0.62 g respectively. The gut evacuation experiment revealed that 50% of the gut was emptied within 24 h in summer while it was 36 h for winter. 86% of the gut was emptied within 72 h in summer, and 75% of the gut emptied in winter (Figure 5.4). The total time for gut evacuation was 100h in winter, while it emptied within 33.3 h in summer.

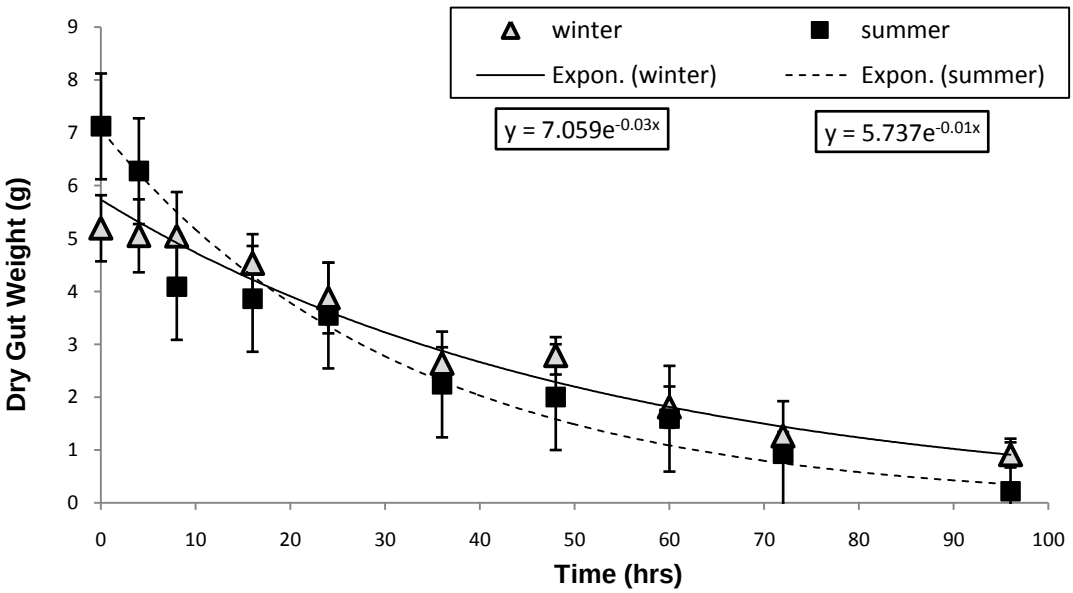

Figure 5.4 Gut evacuation of *C. tenuispinus* over 96h in winter (n = 50) 2015 and summer 2016 (n = 50), Exponential curve fitted by least regression on the natural logarithms of dry gut weight with time.

Gut evacuation rate was 0.72 g day^{-1}and 0.24 g day^{-1} for summer and winter respectively. Estimated gut turnover rates for autumn and spring was 0.70 g day^{-1} and 0.48 g day^{-1} (Table 5.3).

Table 5.3 Gut turnover rate, food ingestion rate and grazing rate of *C. tenuispinus* (mean ± SE, n = 364).

Season	Gut turnover rate (g day^{-1})	Food ingestion rate (gut dry weight individual^{-1} day^{-1})	Grazing rate (gut dry weight individual^{-1} day^{-1})
Summer-2015	0.72	4.67±0.95	0.58±0.18
Autumn-2015	0.70	4.58±0.96	0.48±0.14
Winter-2015	0.24	1.62±0.39	0.14±0.05
Spring-2015	0.48	3.25±1.06	0.31±0.10
Summer-2016	0.72	5.02±1.32	0.50±0.13

Strong variations were observed in food ingestion rates with respect to seasonality ($F_{4,364)} = 180.999$, $p < 0.001$); however, food ingestion rates between summer 2015 and autumn 2015 ($p = 0.981$) and summer 2015 and summer 2016 were similar. Differences in food ingestion rates varied among the three size classes ($F_{(2,364)} = 47.460$, $p < 0.001$). Mean food ingestion rates for the size classes were 3.33 ± 1.28 g day^{-1} (< 65 mm), 3.80 ± 1.46 g day^{-1} (65 - 70 mm), and 4.25 ± 1.79 g day^{-1}(> 70 mm).

5.3.1.4 Analysis of sea urchin density

Population density varied from 3.20 ± 0.64 m^{-2} to 3.73 ± 1.03 m^{-2} throughout the study period (Table 5.4).Test diameters for individual samples were 51.24 - 82.35 mm.

No significant differences were observed in test size and sea urchin density between seasons ($F_{(4,364)} = 0.823$, $p = 0.524$).

Table 5.4 Mean test diameter (mean ± SD, n = 364) and density of *C. tenuispinus*.

Season	Test diameter (mm)	Mean sea urchin density (individuals m^{-2})
Summer-2015	67.70±5.95	3.33±0.64
Autumn-2015	67.79±6.76	3.45±0.93
Winter-2015	68.61±6.47	3.65±0.95
Spring-2015	67.46±5.75	3.73±1.03
Summer-2016	69.06±5.37	3.20±0.64

5.3.5 $CaCO_3$ ingestion rates and seasonal bio-erosion rates

Calcium carbonate ingestion rates varied between seasons and size classes ($p < 0.001$). The interaction between season and size was also significant ($F_{(8,364)} = 2.322$, $P = 0.019$). Pair-wise comparisons revealed that there were no significant differences between $CaCO_3$ ingestion rates in summer 2015 (3.92 ± 0.83 g $CaCO_3$ day^{-1}) and autumn 2015 (3.97 ± 0.85 g $CaCO_3$ day^{-1}). Highest $CaCO_3$ ingestion rate was inferred in summer 2016 (4.36 ± 1.18g $CaCO_3$ day^{-1}) while lowest ingestion rate was observed in winter 2015 (1.41 ± 0.34g $CaCO_3$ day^{-1}) (Table 5.5). $CaCO_3$ ingestion rates for size classes < 65mm, 65 - 70mm and > 70mm were 2.84 ± 1.09g $CaCO_3$ day^{-1}, 3.28 ± 1.26 g $CaCO_3$ $day^{-1}$and 3.67 ± 1.54 g $CaCO_3$ day^{-1} respectively. Large urchins had the highest $CaCO_3$ ingestion rates.

Mean seasonal new $CaCO_3$ erosion was 21.30 ± 3.23% (mean of 1.46 ± 0.44 g $CaCO_3$). The proportion of reworked $CaCO_3$ was 64.99% (mean of 4.44 ± 1.11 g $CaCO_3$), which was nearly three times the rate of new erosion. Summer 2015 had the lowest proportion of newly eroded $CaCO_3$, and this was significantly different from the other seasons. The extent of new erosion was similar between other seasons (Table 5.5).

Overall, individual bio-erosion rates ranged from 0.19 - 2.12 g $CaCO_3$ day^{-1}. Individual bio-erosion rates for summer 2015, autumn 2015, winter 2015, spring 2015 and summer 2016 were 0.4 - 1.49 g $CaCO_3$ day^{-1}, 0.47-1.57 g $CaCO_3$ day^{-1}, 0.19-0.56 g $CaCO_3$ day^{-1}, 0.29-1.45 g $CaCO_3$ day^{-1}, 0.41 - 2.12 g $CaCO_3$ day^{-1} respectively.

Table 5.5 CaCO3 ingestion rate, percentage newly eroded CaCO3 and bio-erosion rate of *C. tenuispinus* (mean ± SD, n = 364).

Season	$CaCO_3$ ingestion rate (g $CaCO_3$ $individual^{-1}day^{-1}$)	Newly eroded $CaCO_3$ percentage	Bio-erosion rate (g $CaCO_3$ $individual^{-1}day^{-1}$)
Summer-2015	3.92±0.83	18.83±3.72	0.88±0.26
Autumn-2015	3.97±0.85	21.60±2.36	0.99±0.24
Winter-2015	1.41±0.34	22.30±2.69	0.36±0.09
Spring-2015	3.06±0.85	22.19±2.73	0.78±0.24
Summer-2016	4.36±1.18	21.67±2.96	1.10±0.36

The highest bio-erosion rate (1.1 ± 0.36 g $CaCO_3$ individual^{-1} day^{-1}) was recorded in summer 2016, and the lowest (0.36 ± 0.09 g $CaCO_3$ individual^{-1} day^{-1}) was recorded in winter 2015 (Table 5.5). Daily bio-erosion rates were significantly different between seasons and the size classes (Table 5.6). No significant differences were observed between bio-erosion rates of autumn 2015 and summer 2016 (p = 0.077).

Table 5.6 Results of two-way ANOVA on bio-erosion rates, season and test size as factors (n = 409), (α = 0.05). * denotes significant results.

	Type III corrected Sum of Squares	df	Mean Square	F	Sig.
Corrected model	29.692	14	2.121	39.135	<0.001
Intercept	210.891	1	210.891	3891.374	<0.001
Season	22.020	4	5.505	101.580	<0.001*
Test size	4.096	2	2.048	37.789	<0.001*
Season*Test size	0.708	8	0.88	1.632	0.114
Error	18.914	349	0.54		
Total	281.320	364			
Corrected Total	48.606	363			

Bio-erosion rate was significantly lower in small size class (< 65 mm) irrespective of the season (Figure 5.5). Aristotle's lantern was smaller in small sized urchins compared to larger urchins (Table 5.7).

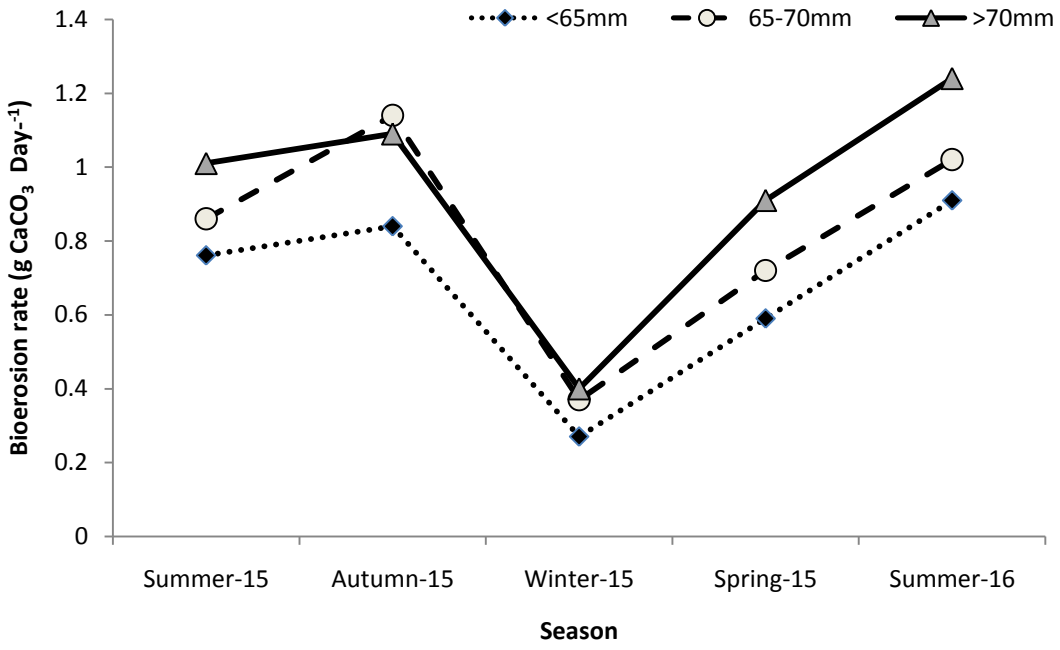

Figure 5.5 Seasonal variations in bio-erosion rates of three test size classes of *C. tenuispinus* (n = 364) in Hall Bank reef.

Table 5.7 Lantern weight, Lantern diameter and Lantern jaw height of *C. tenuispinus* in Hall Bank reef (mean ± SD), n = 364.

Urchin size	Small	Medium	Large
Lantern weight /g	6.10±1.08	7.57±1.38	10.40±1.78
Lantern diameter /mm	21.19±1.56	23.37±1.74	26.20±2.20
Lantern jaw height /mm	21.80±1.61	23.84±1.76	27.10±2.17

The highest seasonal bio-erosion (316.80 g $CaCO_3$ m^{-2}) was recorded in summer 2016 and the lowest recorded in winter 2015 (121.44g $CaCO_3$ m^{-2}) (Table 5.8). The annual bio-erosion rate calculated for Hall Bank Reef from this study was 1017.69 g $CaCO_3$ m^{-2} y^{-1}.

Table 5.8 Mean daily bio-erosion, seasonal bio-erosion and annual bio-erosion in Hall Bank Reef

Season	Daily bio-erosion g of $CaCO_3$ day^{-1} m^{-2}	Seasonal erosion g of $CaCO_3$ m^{-2}	Mean seasonal erosion g of $CaCO_3$ m^{-2}	Annual bio-erosion g of $CaCO_3$ yr^{-1} m^{-2}
Autumn-15	3.42	314.64	104.88	1017.69
Winter-15	1.32	121.44	40.48	
Spring-15	2.91	264.81	88.27	
Summer-16	3.52	316.80	105.60	

5.3.6 Impact of temperature on bio-erosion rates

Seawater temperature was positively correlated with bio-erosion rates at Hall Bank Reef (Pearson correlation = 0.831, p = 0.006) (Figure 5.6). Winter 2015 had the lowest bio-erosion rates and summer 2016 had the highest rates. The mean temperature in summer 2015 was 1° C colder (22.51 °C) than summer 2016 (23.52 °C).

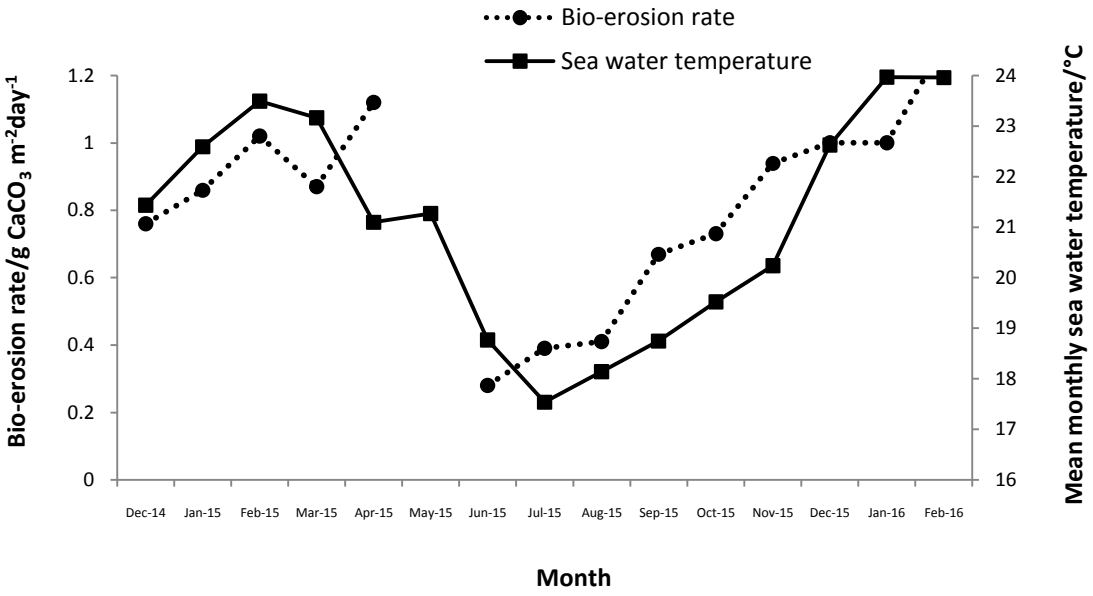

Figure 5.6 Mean monthly variations in mean seawater temperature (°C) and bio-erosion rates (g $CaCO_3$ m^{-2} day^{-1}) of *C. tenuispinus* in Hall Bank reef from.

5.4 Discussion

Sea urchins are well known for their ability to alter reef structure and produce sediment in many shallow marine benthic reefs (Bak 1994; Glynn 1988), mainly associated with their feeding mechanism and behaviours. Urchin food ingestion rate and bio-erosion rate are mainly influenced by urchin species, test size, and population density (Bak 1994). Being herbivores, some urchin species depend on drifting algae, while others actively graze on attached algae. As a result of active grazing, hard substrate particles are removed, altering the reef structure. Gut contents of urchins reflect the types of food mostly available in the habitat (Chapter 2 / Chapter 4). Differences in proportions of inorganic and organic contents in the alimentary canals of these scrapers and browsers have been documented in studies on *Echinometra mathaei*, *Diadema setosum*, *Diadema savignyi* and *Echinothrix diadema* in Kenyan reefs (Carreiro-Silva and McClanahan 2001). The current study revealed that *Centrostephanus tenuispinus* gut contents were mainly composed of inorganic components (89.68 ± 2.77 %). The inorganic portion of the gut contents included calcareous rock and sand fragments. Vanderklift et al. (2006) recorded similar results in their study on *C. tenuispinus* in south-Western Australia. This study confirms that *C. tenuispinus* is an active grazer.

Calcium carbonate was the main constituent of the inorganic gut contents (mean 86.30 ± 3.23 %). Similar studies elsewhere have documented similar outcomes, having higher percentages of calcium carbonates with respect to organic component (Brown-Saracino et al. 2007; Carreiro-Silva and McClanahan 2001). Other diadematoid urchins *Diadema setosum*, *Diadema savignyi* and *Echinothrix diadema* known to have over 80 % of calcium carbonate in their diets (Carreiro-Silva and McClanahan 2001). Daily calcium carbonate consumption was five times that of organic component consumption of these tropical sea urchins (Carreiro-Silva and McClanahan 2001). In this study, daily consumption of calcium carbonate by *C. tenuispinus* was eight times higher than consumption of organic components. The low proportion of organic material in urchin's guts suggests that this herbivore assemblage is food-limited. Hall Bank Reef lacks established macroalgae beds, probably due to competition for space from corals and intensive grazing of any algal recruitment. Urchins therefore totally depend on turf algae and occasional drifting algae / seagrass.

Based on stable isotope analysis, *C. tenuispinus* is an omnivore which feeds on both algae and animal matter (Vanderklift et al. 2006) (Chapter 4). Analysis of gut contents in this study confirms that *C. tenuispinus* feeds on both turf algae and other

invertebrates (Chapter 4). Thus, the organic component in the gut corresponds not only to the grazed algae but also to the invertebrate tissues. Urchins from the reworked calcium carbonate experiment had a higher percentage of organic components (29.71 ± 1.44 %), mainly due to the availability of seagrass. Most of the $CaCO_3$ components were derived from encrusting coralline algae and the coral substrate. Mollusc shells (snails and bivalves) were also noted during the analysis. Since it was difficult to separate these minute shells from rest of the gut contents, calcium carbonate contribution of shells was not quantified separately. Thus, a small portion of these animal shells also might contribute to the total weight of calcium carbonates. The presence of siliceous material (spicules) indicates urchins were also feeding upon other invertebrates such as sponges and bryozoans.

Higher proportions of inorganic gut contents in medium and larger urchins suggest these urchins have the ability to graze more intensively over the substrate. Large urchins had significantly heavier and larger Aristotle's lanterns compared to small urchins. Urchin tests and lanterns get more calcified and heavy with age as well. Thus, large urchins are capable of more intense scraping. Small urchins have small lanterns and tend to graze more on attached algae than the substrate. Higher dry gut weight in large size classes reflects their ability to feed more compared to small size classes. High food ingestion rates with respect to larger sizes are well described by Kawamata (1997) for urchin *Strongylocentrotus nudus*. The importance of urchin species and body size on grazing has also been demonstrated by Carreiro-Silva and McClanahan (2001) as well. Similarly, a positive correlation between test diameters and the extent of bio-erosion has been witnessed for *Echinometra mathaei* (> 30 mm, 166.70 mg day^{-1}; < 30 mm, 77.78 mg day^{-1}) in Japan (Manullang et al. 2014) and *Diadema antillarum* (< 30 mm, 0.54 ± 0.45 g / > 50 mm, 3.88 ± 0.74 g) in western Mexico (Herrera-Escalante et al. 2005). *Centrostephanus tenuispinus* in this study ingests 6.83 ± 1.70 g of food daily. This study confirms the positive correlation of test size on the level of bio-erosion as well.

Previously conducted studies on bio-erosion were mainly focused on tropical urchins, mainly *Diadema*, *Echinometra* and *Echinothrix* species, and these species are considered to be the most impacting bio-eroding agents in tropical reefs (Bak 1990; Mokady et al. 1996). Environmental conditions, particularly water temperature, impact the urchins' physiology, directly influence ingestion and gut evacuation rates. Since there were no significant differences in dry gut weight across the seasons sampled in this study, it is clear that differences in ingestion rate are critical for determining the rate of bio-erosion. Significant differences in seasonal ingestion rates are in response to

seawater temperature changes, from 17 °C in winter to 22 °C in summer. Food ingestion rates were higher in summer due to increased metabolism at the higher temperatures. Thus, the bio-erosion rates were higher as well. *Centrostephanus tenuispinus* in the current study was subjected to lower water temperatures (17°C) in winter than summer (23 °C). A difference of 6 °C directly affected physiology and activity levels and therefore changes in ingestion rates between winter and summer. The ingestion rate for summer 2015 is lower than that of summer 2016. The highest ingestion rate was recorded in summer 2016 (5.02 ± 1.33 g individual^{-1} day^{-1} / 23 °C). Increase in feeding rates from 26.35 ± 3.37 mg C day^{-1} to 40.38 ± 3.5 mg C day^{-1} has been recorded for *Lytechinus variegatus* in Florida, in response to increasing temperature from 20 °C to 27 °C (Lemoine and Burkepile 2012). Lower feeding rates in winter in response to sea water temperature has also been recorded for *Strongylocentrotus nudus* in Japan (Kawamata 1997). Most urchin species in the tropics experience water temperatures over 25 °C all round year, and therefore have consistently high ingestion rates. Since the rate of bio-erosion is based on food ingestion rates, any factor affecting food intake indirectly affects the bio-erosion rate as well. *Echinothrix diadema* from tropical waters has a mean ingestion rate of 21.09 ± 3.52 g gut dry weight individual^{-1} day^{-1} (Carreiro-Silva and McClanahan 2001), which is higher than that of *C. tenuispinus* 5.02 ± 1.33 g individual^{-1} day^{-1}. At the same time, the smaller *Diadema setosum* has shown ingestion rate of 8.36 ± 1.16 g day^{-1} (Carreiro-Silva and McClanahan 2001). The influence of water temperature on feeding has been recorded for other diadematoids (Coppard and Campbell 2005a), and slower food ingestion rates at low temperatures have been observed in many fish species and turtles (Handeland et al. 2008; Parmenter 1980).

High inorganic and low organic content in winter 2015 indicates low food availability in winter. Many studies suggest that urchin feeding is affected by reproductive periods (Fuji 1962; Lawrence 2013; Muthiga 2003a). Some urchin species feed less during spawning periods; *Strongylocentrotus intermedius* rarely feed during reproductive season (Fuji 1962). *C. tenuispinus* tends to spawn during the winter with an extended spawning period up to the end of spring (Chapter 3). Lowest food ingestion rates were observed in winter (1.62 ± 0.39 g dry weight day^{-1}) and spring (3.51 ± 0.97 g dry weight day^{-1}), which is thought to be directly related to sea water temperatures (Kawamata 1997). The lowest proportion of organic components in urchin's guts was recorded in winter 2015 (8.74 ± 1.77 %) when urchins were spawning. Summer 2015, autumn 2015 and summer 2016 had higher proportions of organic components; this is when urchin gonads are in the resting phase and initiating gametogenesis of next cycle

(Chapter 3). *Strongylocentrotus droebachiensis* is known to feed more at high temperatures, favouring the production of nutritive phagocytes (Garrido and Barber 2001). Similarities between food ingestion rates in autumn and summer are thought to be due to higher water temperatures in these seasons compared to winter 2015 and spring 2015.

Diadema savignyi, *Diadema setosum* and *Echinothrix diadema*, are diadematoids known to greatly influence many tropical reefs. These urchins are similar in size and feeding habits to *C. tenuispinus*. *C. tenuispinus* in this study had similar dry gut weight (spring 2016 = 7.31 ± 2.02 g / summer 2015 = 6.48 ± 1.33 g) to *Diadema setosum* (mean dry gut weight = 7.05 ± 0.98 g), possibly due to their similar range of body size (Table 5.9). Although *Diadema savignyi* is similar in size to *Diadema setosum* and *Centrostephanus tenuispinus*, it ingests less food (mean dry gut weight = 3.48 ± 0.78 g) (Carreiro-Silva and McClanahan 2001). *Diadema savigynyi* and *D. setosum* are known to coexist in the same habitat (Muthiga 2003b). As Carreiro-Silva and McClanahan (2001) suggested, despite having similar test diameters, differences in body physiology and species specific feeding behaviours can impact the extent of feeding.

Table.5.9 Test diameter, mean dry gut weight, food ingestion rates, gut turnover rates, daily bio-erosion rates of sea urchins *Diadema setosum*, *D. savignyi*, *Echinothrix diadema* and *C. tenuispinus* (mean ± SD).

Species	***Diadema setosum*** Carreiro-Silva and McClanahan (2001)	***Diadema savignyi*** Carreiro-Silva and McClanahan (2001)	***Echinothrix diadema*** Carreiro-Silva and McClanahan (2001)	***Centrostephanus tenuispinus*** **(This study/summer)**
Test diameter (mm)	67.74 ±0.88	69.86±0.98	103.96±1.02	**69.06±0.61**
Mean gut weight(g)	7.05±0.98	3.48±0.78	18.54±3.10	**6.98±0.21**
Food ingestion rate (gut dry weight individual^{-1} day^{-1})	8.34±1.16	3.11±0.70	21.09±3.52	**5.02±0.15**
Gut turnover rate (day^{-1})	1.18	0.89	1.14	**0.72**
Bio-erosion rate (g $CaCO_3$ individual^{-1} day^{-1})	1.79±0.25	0.72±0.16	5.49±0.91	**1.10±0.04**

Gut evacuation rate for *Centrostephanus tenuispinus* was nearly 100 hours in winter, which is longer than other sea urchin species, while summer gut evacuation rate was 33.3 hours. Carreiro-Silva and McClanahan (2001) reported that the common tropical diadematoids *Diadema setosum*, *D. savignyi* and *Echinothrix diadema* take 20.29, 26.86 and 21.10 hours respectively to empty their guts. *Echinometra mathaei*

takes 13.94 hours to empty all gut contents (Carreiro-Silva and McClanahan 2001). The slower body physiology recorded for *C. tenuispinus* in this study may be associated with low winter temperatures; *C. tenuispinus* inhabits colder waters compared to other diadematoids studied in this regard.

Sea urchin feeding, and hence bio-erosion rate, is influenced by space, nature of the habitat, habitat structure and water temperature. Interaction of these factors causes higher bio-erosion in some habitats. Bio-erosion rates witnessed in this study were mainly dependent on three factors: the amount of food ingested (dry gut weight), gut content composition, and food ingestion rate. The amount of food ingested was found to be dependent on the size of urchins. A seasonal impact on dry gut weight was observed only between spring 2015, summer 2015 and autumn 2015. Significant differences in food ingestion rates and bio-erosion rates between summer 2015 and summer 2016 were mainly depended on differences in gut weight and percentage of $CaCO_3$. Further, summer 2015 was 1 °C colder than summer 2016.

Among other factors, the density of the urchin population has a significant impact on structuring benthic communities, and therefore the extent of reef bio-erosion (Hereu et al. 2004; Ling and Johnson 2009). High densities of urchins can cause higher levels of bio-erosion (Bak 1994; Eakin 1996; Glynn et al. 2017; Glynn 1988; McClanahan and Kurtis 1991). *Eucidaris galapagensis* on the reef flats of Floreana Island, and Galapagos Islands are known to contribute to reef erosion of 3,320 g $CaCO_3$ m^{-2} y^{-1} and 22,332 g $CaCO_3$ m^{-2} y^{-1} with population densities of 4.6 individuals m^{-2} and 30.8 individuals m^{-2}, respectively (Glynn 1988). High densities of small urchins can have a larger bio-erosion impact than low densities of larger individuals (Griffin et al. 2003). *Diadema mexicanum* in Panama is known to cause bio-erosion of 139-277 g $CaCO_3$ m^{-2} y^{-1} at densities of 2 - 4 individuals m^{-2} and 3,470 - 10,400 g $CaCO_3$ m^{-2} y^{-1}at densities of 50 - 150 individuals m^{-2}. *E. mathaei* at Ningaloo Reef has been reported to erode 980 – 4550 g $CaCO_3$ m^{-2} y^{-1} at densities of 10.8 - 17.4 individuals m^{-2} (Langdon 2012). *C. tenuispinus* in the current study is responsible for bio-erosion of 1,017.69 g $CaCO_3$ m^{-2} y^{-1}at densities of 3.2 - 3.7 individuals m^{-2}, which is higher than *D. mexicanum* at similar densities.

The reef accretion rate of Hall Bank Reef has not been quantified. Coral calcification rates are known to depend on sea water temperatures (Kleypas et al. 1999). Hall Bank reef is dominated by massive corals (Chapter 2). Slower growth rates were recorded for the massive coral *Coelastrea aspera* in Marmion Reef compared to its tropical counterpart after the influence of heat wave (2011 – 2013) (Foster et al. 2014) .

Since Marmion Reef is in close proximity to Hall Bank reef and having similar sea water temperature ranges (22 °C – 23 °C), calcification rate in Marmion Reef is assumed to be similar in Hall Bank reef. Highest calcification rates were recorded for Marmion for winter 2011 (7.3 g m^{-2} day^{-1}) after the 2011 heat wave, which is higher than daily bio-erosion rates in Hall Bank reef (Foster et al. 2014). However, calcification rates for summer 2012, winter 2012 and summer 2013 was low (1 – 2.5 g m^{-2} day^{-1}), which is lower than daily bio-erosion rates of Hall Bank reef in both summer and autumn. However, mean annual growth rates recorded for *Coelastrea aspera / Goniastrea palauensis* and *Paragoniastrea australensis* in Hall Bank reef are 5.4 ± 0.9 mm a^{-1} and 10.9 mm a^{-1} (Antipas 2013). Higher growth rates of these corals Hall Bank reef, despite its near shore location and compared to other tropical/subtropical locations, could be attributed to increasing trends of sea water temperature. Lack of seasonality in calcification rates and slower growth of branching corals compared to massive corals is known to be caused by temperature anomalously occurred in 2010 - 2011 (Foster et al. 2014). Since bio-erosion is positively correlated with sea water temperature, increase in sea water temperature could also lead to conditions where reef erosion rates exceed reef accretion rates, which can affect the coral cover in Hall Bank reef. It is essential to calculate daily calcification rates to properly understand reef carbonate budgets in Hall Bank reef.

In most marine benthic habitats, the population density of particular species is controlled by predation (top-down control) and food availability (bottom-up control) (Dee et al. 2012; McClanahan et al. 1996; O'Leary and McClanahan 2010; Sheppard-Brennand et al. 2016). Many studies have suggested that fish predators exert strong top-down control on urchins (Hughes et al. 1987; McClanahan 1997; McClanahan 1999b). Overfishing has impacts on urchin population in two ways: by removal of predators and removal of competitors. Urchin abundance has been observed to be inversely related to the densities of fish from the family Labridae (Brown-Saracino et al. 2007). Previous studies have revealed that management status (protected/unprotected) of a marine habitat is also important (Brown-Saracino et al. 2007; Carreiro-Silva and McClanahan 2001).

Bio-erosion resulting from spine abrasion has not been accounted for in this study since it was difficult to quantify under field conditions. Similarly, bio-erosion caused by other herbivores has not been accounted for, since *C. tenuispinus* is considered to be the main bio-eroder in this system. This study indicates that the difference in food ingestion rates in response to seawater temperature changes is the

main cause for the significant differences in seasonal bio-erosion rates in Hall Bank Reef. Seasonal changes in seawater temperature in Hall Bank Reef not only influence the extent of sea urchin feeding but also reef health.

Chapter 6 - General discussion and conclusions

The critical role of sea urchins in structuring many marine benthic habitats has been well recognized in both tropical and temperate geographical regions around the world (Adam et al. 2015; Carpenter 1990; Carpenter 1988; Forcucci 1994; Hill et al. 2003; Lawrence 2013; Lessios 1988a; Lessios 1995; Scheibling and Stephenson 1984). This study was focused on filling knowledge gaps in key biological and ecological aspects of sea urchin *Centrostephanus tenuispinus* and evaluating its role in Hall Bank reef. The study was structured on the following aspects;

1. Population density and size distribution and with respect to food availability (Chapter 2)
2. Reproductive biology (Chapter 3)
3. Feeding ecology (Chapter 4)
4. Bio-erosion (Chapter 5)

The comprehensive study of population size structure, reproductive biology, feeding ecology and bio-erosion of *Centrostephanus tenuispinus* provided baseline data needed for managing reefs with this particular species. *Centrostephanus tenuispinus* exhibited many similarities with its congener *C. rodgersii* in Eastern coast of Australia, in terms of reproductive patterns, population size structure and feeding ecology. *Centrostephanus rodgersii* is well known for its recent range expansion into Tasmanian waters as a result of climate change, and its capability of establishing in its new habitat, influencing its species composition and structure (Ling 2008; Ling et al. 2008). With such high adaptability, *Centrostephanus tenuispinus* also has the potential to survive in new environments. The second chapter of this thesis presented the capability of *Centrostephanus tenuispinus* in shifting feeding behaviours with different levels of food availability and a diversity of food sources.

Hall Bank reef is unique in the region due to its high coral cover, absence of macroalgae, soft coral and a high density of *C. tenuispinus* (Thomson and Frisch 2010). *Centrostephanus tenuispinus* populations was studied at Hall Bank, a structurally simple, low productive habitat (dominated by turf algae), and Minden Reef, a structurally complex, highly productive habitat (dominated by macroalgae). Comparisons were made with respect to reproductive output, population structure and feeding ecology.

As illustrated in Chapter 2, the main contributors of substrate cover on Hall Bank reef were turf and crustose coralline algae. On the other hand, large brown foliose

algae and seagrass were important contributors to Minden Reef substrate cover. In contrast to low seasonal variability in substrate cover of Hall Bank, marked seasonal changes were observed at Minden Reef, from *Sargassum* dominance (winter and spring) to *Dictyopteris*, turf algae and sponge dominance (summer and autumn). The observed variations in feeding ecology and reproductive output of *C. tenuispinus* at Minden Reef were attributed to high seasonal variability of substrate cover. Although coral cover at Hall Bank reef was high compared to Minden Reef, it was only one fourth of the coral cover recorded by Thomson and Frisch (2010). The decrease in coral over the past few years correlated with increased sponge cover and rubble. An experiment using exclusion cages revealed that *C. tenuispinus* is responsible for controlling the benthic algae composition at Hall Bank reef within a short period of time, exhibiting similarities with other sea urchin species elsewhere. Grazing by *C. tenuispinus* favoured higher species diversity and dominance of crustose coralline algae. Similar observations have also been recorded in geographical regions with sea urchin barrens (Bonaviri et al. 2011; Filbee-Dexter and Scheibling 2014; Flukes et al. 2012; Valentine and Johnson 2005). Thus, the loss or removal of *C. tenuispinus* from Hall Bank can be assumed to lead to extensive growth of benthic algae.

High population density of *C. tenuispinus* with smaller test sizes at Hall Bank reef indicated low food availability, while lower urchin densities with larger test diameters at Minden Reef indicated high productivity in the habitat, as reported elsewhere (Ling and Johnson 2009). Although significant differences in mean test diameters were observed at the two sites, population structure showed a similar pattern of unimodal test size frequency distribution. The absence of dominant predators on urchins at both study sites could also responsible for the unimodal size distribution (Shears and Babcock 2002; Tuya et al. 2004). Predation levels and growth rates are also needed for the better understanding of size structure in two reefs; which is a limitation of this study. The absence of small individuals in the present study could be due to two reasons; either cryptic behaviour of small urchins or low recruitment/settlement. Poor recruitment for four consecutive years in three *Centrostephanus rodgersii* populations in New South Wales, resulted in a lack of small individuals (< 60 mm) (Andrew and Underwood 1989a). Since successful spawning events occurred during three consecutive years in this study, a possible reason for absence of small individual could be their cryptic behaviour. Although small individuals are scarce at Minden Reef, once urchins emerge from their cryptic nature, they are likely to have high growth rates due to the high availability of food. They therefore attain large sizes within a short period of

time. In contrast, urchins in Hall Bank reef maintain a stationary growth mode after reaching a certain size, in response to the low food availability (Pecorino et al. 2012). Higher Aristotle's Lantern Index values in urchins from Hall Bank reef confirmed the overall scarcity of food at that site. The co-existence of other urchin species with similar feeding habits at Minden Reef reflected a higher diversity and abundance of available food sources in the habitat.

Chapter 3 discussed the reproductive biology of the *C. tenuispinus* population at Hall Bank reef. *C. tenuispinus* exhibited a clear annual reproductive cycle and similar trends with the reproductive cycle of *C. rodgersii* were witnessed (Byrne et al. 1998; King et al. 1994; Pecorino et al. 2013a). Male and female cycles were synchronized. The reproductive cycle consisted of developing, premature, mature, partially spent, spent, and recovery stages, which follows a similar series of cellular events as *C. rodgersii* and other diadematoid sea urchins (Byrne et al. 1998; Drummond 1995; King et al. 1994; Pecorino et al. 2013a). The gametogenic cycle was initiated in March (onset of autumn), with decreasing sea water temperature and decreasing day length. Gametes tend to have a short maturing phase (1 month) and spawning is initiated in July-August. A prolonged spawning period of five months was observed in *C. tenuispinus*. Although the Gonadosomatic Index (GSI) does not show correlation with sea water temperatures or day length, histological observations of the cellular process coincide with the lower sea water temperature and decreasing day length.

This study revealed that *C. tenuispinus* is omnivorous and their diet is composed of a considerable amount of animal tissues (discussed in Chapter 4). High nutritional values of food in autumn could also be a precursor for initiating gametogenesis in autumn. The high amount of animal tissue in their diet is mainly due to high availability of animal prey in the habitat. Feeding is known to impact reproductive output and growth of urchins (Byrne et al. 1998). Despite having similar reproductive periodicity, Minden Reef urchins had a higher GSI, coinciding with high food availability.

The feeding ecology of *C. tenuispinus* at the two study sites was presented in Chapter 4. Results of gut content analysis revealed that *C. tenuispinus* is omnivorous and an active grazer, feeding on attached benthic species. The higher trophic level values of *C. tenuispinus* derived from stable isotope analysis further confirmed their omnivorous nature (Vanderklift et al. 2006). The higher amount of $CaCO_3$ in the diet of urchins from Hall Bank reef, compared to Minden Reef, was due to two reasons: intense grazing on attached forms due to low food availability, and higher availability of coral and coralline substrates at Hall Bank reef. The diet of Hall Bank urchins was composed

of turf algae (mainly *Polysiphonia* sp.), foraminiferans and spicules. Further, the diet of urchins mainly consisted of the same species recorded in open settlement plates, which indicates the substrate was subjected to constant intense grazing; *Polysiphonia* sp. being the most abundant food source in the grazed substrate therefore contributed continuously to diets. Further, differences in species composition between open and sea urchin exclusion plates indicates the impact of *C. tenuispinus* grazing on benthic species composition. Although the exclusion plates were only left on the reef for one month due to the high exposure to currents, within this short period plates were rapidly colonised by filamentous brown algae. Dominance of filamentous algae followed by macroalgae dominance has been witnessed in barrens habitats with *Centrostephanus rodgersii* exclusions in eastern Australia (Hill et al. 2003). Shifts from coralline algae dominance to macroalgae dominance have been observed to result from sudden mass mortality of *Diadema antillarum* in the Caribbean and *Strongylocentrotus* in the North Atlantic (Carpenter 1990; Lessios 1988b; Lessios et al. 1984a; Scheibling and Stephenson 1984). Crustose coralline algae are known to contribute $CaCO_3$ in the diet; however, the percentage of crustose coralline algae in urchin diets was not able to be quantified in this study. High seasonal variability in feeding rates was correlated with changes in sea water temperature; however, low seasonal variability in diet composition of urchins at Hall Bank reef was associated with less variability in substrate cover. Conversely, the pronounced seasonal variation in diet composition recorded for Minden Reef urchins was correlated with seasonal changes of substrate cover. Higher $CaCO_3$, low macroalgae and high turf algae in diets of *C. tenuispinus* indicated shifts of their feeding habits in summer.

Chapter 5 discussed the role of *C. tenuispinus* as a bio-eroder in Hall Bank reef. Bio-erosion associated with feeding was quantified during this study. Seasonal changes in food ingestion rates could be influenced by sea water temperatures and changes in food availability. Thus, seasonal variations in bio-erosion rates recorded during this study. Higher bio-erosion rates in larger urchins were due to the larger Aristotle's lantern and more intense grazing, compared to smaller urchins. The highest seasonal rate of bio-erosion was recorded in summer 2016, and positively correlated with sea water temperatures. The annual bio-erosion rate calculated for Hall Bank reef from this study was 1017.69 g $CaCO_3$ m^{-2} y^{-1}. Lower calcification rates for the dominant coral, *Goniastrea* sp. (1-2.5 g $CaCO_3$ $m^{-2}day^{-1}$) (winter 2011-summer 2013), was recorded for nearby Marmion Reef after the heat wave in 2010-2011 (Foster et al. 2014); this rate was lower than the erosion rates (3.41-3.52 g $CaCO_3$ $m^{-2}day^{-1}$) recorded for autumn and

summer in Hall Bank in the present study. Although calcification rates have not been estimated for Hall Bank reef, erosion rates exceeding the calcification rates of reefs in the region indicates the likely high impact of urchin grazing at Hall Bank reef.

In conclusion, *C .tenuispinus* is an essential component of the Hall Bank reef system. Being the main grazer and bio-eroder at Hall Bank reef, changes in their population can lead to larger impacts on the structure and composition of benthic cover of the reef. Although an absence of urchins at Hall Bank could decrease reef erosion, excess growth of algae could lead to a reduction in coral cover. With sea urchin exclusion, succession of filamentous algae followed by macroalgae is likely. On the other hand, a sudden increase in urchin populations in macroalgae dominated reefs such as Minden Reef could drive the habitat to a barrens state, influencing the species composition of the reef, as observed with its congener, *Centrostephanus rodgersii*. The current trends in changing climate, rising sea water temperature, eutrophication and changes in ocean chemistry can lead to dramatic changes in reef environments, greatly influencing urchin populations. It is essential to study the larval ecology and settlement processes of *C. tenuispinus* to understand recruitment success in both macroalgae-dominated reefs and barrens. Knowledge on reproductive biology will be potentially important in case of developing *C. tenuispinus* fisheries in Western Australia and predicting harvesting regimes. Most importantly, comprehensive knowledge acquired through this study on population size structure, reproductive biology, feeding ecology and bio-erosion of *Centrostephanus tenuispinus* provides baseline data for the region on this particular species which could be used in monitoring and managing reefs with current trends in climate change.

List of References

Abdo DA, Bellchambers LM, Evans SN (2012) Turning up the heat: increasing temperature and coral bleaching at the high latitude coral reefs of the Houtman Abrolhos Islands PLoS One 7:e43878

Abraham ER (2007) Sea-urchin feeding fronts Ecological Complexity 4:161-168 doi:http://dx.doi.org/10.1016/j.ecocom.2007.06.001

Adam TC, Burkepile DE, Ruttenberg BI, Paddack MJ (2015) Herbivory and the resilience of Caribbean coral reefs: knowledge gaps and implications for management Marine Ecology Progress Series 520:1-20

Agatsuma Y, Hoshikawa H (2007) Northward extension of geographic range of the sea urchin Hemicentrotus pulcherrimus in Hokkaido, Japan Journal of Shellfish Research 26:629-635

Agnetta D, Bonaviri C, Badalamenti F, Scianna C, Vizzini S, Gianguzza P (2013) Functional traits of two co-occurring sea urchins across a barren/forest patch system Journal of sea research 76:170-177

Alemu JB, Clement Y (2014) Mass coral bleaching in 2010 in the southern Caribbean PLoS one 9:e83829

Alsaffar AH, Lone KP (2000) Reproductive cycles of Diadema setosum and Echinometra mathaei (Echinoidea: echinodermata) from Kuwait (northern Arabian Gulf) Bulletin of marine science 67:845-856

Alvarado JJ, Cortés J, Guzman H, Reyes-Bonilla H (2016) Bioerosion by the sea urchin Diadema mexicanum along Eastern Tropical Pacific coral reefs Marine Ecology 37:1088-1102

Alvarado JJ, Cortés J, Reyes-Bonilla H (2012) Reconstruction of Diadema mexicanum bioerosion impact on three Costa Rican Pacific coral reefs Revista de Biología Tropical 60:121-132

Andrew N (1991) Changes in subtidal habitat following mass mortality of sea urchins in Botany Bay, New South Wales Australian journal of ecology 16:353-362

Andrew N et al. (2003) Status and management of world sea urchin fisheries Oceanography and Marine Biology-an Annual Review 40:343-425

Andrew N, MacDiarmid A (1991) Interrelations between Sea-Urchins and Spiny Lobsters in Northeastern New-Zealand Marine Ecology Progress Series 70:211-222

Andrew N, MacDiarmid AB (1999) Sea urchin fisheries and potential ecological interactions with a kina fishery in Fiordland. Department of Conservation,

Andrew N, O'Neill A (2000) Large-scale patterns in habitat structure on subtidal rocky reefs in New South Wales Marine and Freshwater Research 51:255-263

Andrew N, Underwood A (1989a) Patterns of abundance of the sea urchin Centrostephanus rodgersii (Agassiz) on the central coast of New South Wales, Australia Journal of Experimental Marine Biology and Ecology 131:61-80

Andrew N, Underwood A (1993) Density-dependent foraging in the sea urchin Centrostephanus rodgersii on shallow subtidal reefs in New South Wales, Australia Marine Ecology-Progress Series 99:89-89

Andrew NL (1988) Ecological aspects of the common sea urchin, Evechinus chloroticus, in northern New Zealand: a review New Zealand journal of marine and freshwater research 22:415-426

Andrew NL (1993) Spatial Heterogeneity, Sea Urchin Grazing, and Habitat Structure on Reefs in Temperate Australia Ecology 74:292-302 doi:10.2307/1939293

Andrew NL, Byrne M (2007) Ecology of Centrostephanus Developments in aquaculture and fisheries science 37:191-204

Andrew NL, Underwood AJ (1989b) Patterns of abundance of the sea urchin Centrostephanus rodgersii (Agassiz) on the central coast of New South Wales, Australia Journal of Experimental Marine Biology and Ecology 131:61-80 doi:http://dx.doi.org/10.1016/0022-0981(89)90011-7

Antipas KR (2013) Diversity, growth rates and population size structure of a faviid dominated 'marginal' coral reef in Fremantle, Western Australia. Murdoch Univeristy

Appana SD, Vuki VC (2006) Foraging behavior, substrate preference and influence of Echinometra sp. A on the carbonate budget of Nukubuco Reef, Fiji Islands MICRONESICA-AGANA- 38:191
Arafa S, Chouaibi M, Sadok S, El Abed A (2012) The influence of season on the gonad index and biochemical composition of the sea urchin Paracentrotus lividus from the Golf of Tunis The scientific world journal 2012
Asgaard U, Bromley RG (2008) Echinometrid sea urchins, their trophic styles and corresponding bioerosion. In: Current Developments in Bioerosion. Springer, pp 279-303
Bak R (1990) Patterns of echinoid bioerosion in two Pacific coral reef lagoons Mar Ecol Prog Ser 66:272
Bak R (1994) Sea urchin bioerosion on coral reefs: place in the carbonate budget and relevant variables Coral Reefs 13:99-103
Bak R, Carpay M, De Ruyter Van Steveninck E (1984) Densities of the sea urchin Diadema antillarum before and after mass mortalities on the coral reefs on Curacao Marine ecology progress series Oldendorf 17:105-108
Bak RP, van Eys G (1975) Predation of the sea urchin Diadema antillarum Philippi on living coral Oecologia 20:111-115
Baker R, Buckland A, Sheaves M (2014) Fish gut content analysis: robust measures of diet composition Fish and Fisheries 15:170-177
Ban SS, Graham NA, Connolly SR (2014) Evidence for multiple stressor interactions and effects on coral reefs Global Change Biology 20:681-697
Bancroft JD, Gamble M (2008) Theory and practice of histological techniques. Elsevier Health Sciences,
Barnett A, Redd KS, Frusher SD, Stevens JD, Semmens JM (2010) Non-lethal method to obtain stomach samples from a large marine predator and the use of DNA analysis to improve dietary information Journal of Experimental Marine Biology and Ecology 393:188-192 doi:http://dx.doi.org/10.1016/j.jembe.2010.07.022
Barott KL, Rodriguez-Mueller B, Youle M, Marhaver KL, Vermeij MJ, Smith JE, Rohwer FL (2012) Microbial to reef scale interactions between the reef-building coral Montastraea annularis and benthic algae Proceedings of the Royal Society of London B: Biological Sciences 279:1655-1664
Barott KL, Rohwer FL (2012) Unseen players shape benthic competition on coral reefs Trends in microbiology 20:621-628
Bates N et al. (2014) A time-series view of changing ocean chemistry due to ocean uptake of anthropogenic CO2 and ocean acidification Oceanography 27:126-141
Bearhop S, Adams CE, Waldron S, Fuller RA, MacLeod H (2004) Determining Trophic Niche Width: A Novel Approach Using Stable Isotope Analysis Journal of Animal Ecology 73:1007-1012 doi:10.2307/3505535
Beck HJ, Feary DA, Fowler AM, Madin EM, Booth DJ (2016) Temperate predators and seasonal water temperatures impact feeding of a range expanding tropical fish Marine Biology 163:1-14
Beddingfield SD, McClintock JB (1998) Differential survivorship, reproduction, growth and nutrient allocation in the regular echinoid Lytechinus variegatus (Lamarck) fed natural diets Journal of Experimental Marine Biology and Ecology 226:195-215
Beger M, Sommer B, Harrison PL, Smith SDA, Pandolfi JM (2014) Conserving potential coral reef refuges at high latitudes Diversity and Distributions 20:245-257
Behrens MD, Lafferty KD (2004) Effects of marine reserves and urchin disease on southern Californian rocky reef communities Marine Ecology Progress Series 279:129-139
Bell PR, Elmetri I, Lapointe BE (2014) Evidence of large-scale chronic eutrophication in the Great Barrier Reef: quantification of chlorophyll a thresholds for sustaining coral reef communities Ambio 43:361-376

Bellwood D (1996) Production and reworking of sediment by parrotfishes (family Scaridae) on the Great Barrier Reef, Australia Marine Biology 125:795-800
Ben-David M, Flynn R, Schell D (1997) Annual and seasonal changes in diets of martens: evidence from stable isotope analysis Oecologia 111:280-291
Birrell CL, McCook LJ, Willis BL, Diaz-Pulido GA (2008) Effects of benthic algae on the replenishment of corals and the implications for the resilience of coral reefs Oceanogr Mar Biol Annu Rev 46:25-63
Blankenship LE, Yayanos AA (2005) Universal primers and PCR of gut contents to study marine invertebrate diets Mol Ecol 14:891-899 doi:10.1111/j.1365-294X.2005.02448.x
Bluhm BA, Iken K, Hardy SM, Sirenko BI, Holladay BA (2009) Community structure of epibenthic megafauna in the Chukchi Sea Aquatic Biology 7 doi::10.3354/ab00198
Bonaldo RM, Hay ME (2014) Seaweed-coral interactions: variance in seaweed allelopathy, coral susceptibility, and potential effects on coral resilience PLoS One 9:e85786
Bonaviri C, Fernández TV, Badalamenti F, Gianguzza P, Di Lorenzo M, Riggio S (2009) Fish versus starfish predation in controlling sea urchin populations in Mediterranean rocky shores Marine Ecology Progress Series 382:129-138
Bonaviri C, Fernández TV, Fanelli G, Badalamenti F, Gianguzza P (2011) Leading role of the sea urchin Arbacia lixula in maintaining the barren state in southwestern Mediterranean Marine Biology 158:2505
Boolootian RA, Giese A, Tucker J, Farmanfarmaian A (1959) A contribution to the biology of a deep sea echinoid, Allocentrotus fragilis (Jackson) The Biological Bulletin 116:362-372
Boyer K, Fong P, Armitage A, Cohen R (2004) Elevated nutrient content of tropical macroalgae increases rates of herbivory in coral, seagrass, and mangrove habitats Coral Reefs 23:530-538
Bradley BJ, Stiller M, Doran-Sheehy DM, Harris T, Chapman CA, Vigilant L, Poinar H (2007) Plant DNA sequences from feces: potential means for assessing diets of wild primates Am J Primatol 69:699-705 doi:10.1002/ajp.20384
Brady SM, Scheibling RE (2005) Repopulation of the shallow subtidal zone by green sea urchins (Strongylocentrotus droebachiensis) following mass mortality in Nova Scotia, Canada Journal of the Marine Biological Association of the United Kingdom 85:1511-1517
Brett MT, Kainz MJ, Taipale SJ, Seshan H (2009) Phytoplankton, Not Allochthonous Carbon, Sustains Herbivorous Zooplankton Production Proceedings of the National Academy of Sciences of the United States of America 106:21197-21201 doi:10.2307/25593450
Brewin P, Lamare M, Keogh J, Mladenov P (2000) Reproductive variability over a four-year period in the sea urchin Evechinus chloroticus (Echinoidea: Echinodermata) from differing habitats in New Zealand Marine Biology 137:543-557
Brierley AS, Kingsford MJ (2009) Impacts of climate change on marine organisms and ecosystems Current biology 19:R602-R614
Britton-Simmons KH, Foley G, Okamoto D (2009) Spatial subsidy in the subtidal zone: utilization of drift algae by a deep subtidal sea urchin Aquatic Biology 5:233-243
Brodie J et al. (2012) Terrestrial pollutant runoff to the Great Barrier Reef: an update of issues, priorities and management responses Marine Pollution Bulletin 65:81-100
Brogger M, Martinez M, Penchaszadeh P (2010) Reproduction of the sea urchin Arbacia dufresnii (Echinoidea: Arbaciidae) from Golfo Nuevo, Argentina Journal of the Marine Biological Association of the United Kingdom 90:1405-1409
Bronstein O, Kroh A, Loya Y (2016) Reproduction of the long-spined sea urchin Diadema setosum in the Gulf of Aqaba-implications for the use of gonad-indexes Scientific Reports 6
Bronstein O, Loya Y (2014) Echinoid community structure and rates of herbivory and bioerosion on exposed and sheltered reefs Journal of Experimental Marine Biology and Ecology 456:8-17

Bronstein O, Loya Y (2015) Photoperiod, temperature, and food availability as drivers of the annual reproductive cycle of the sea urchin Echinometra sp. from the Gulf of Aqaba (Red Sea) Coral Reefs 34:275-289
Brown-Saracino J, Peckol P, Curran HA, Robbart ML (2007) Spatial variation in sea urchins, fish predators, and bioerosion rates on coral reefs of Belize Coral Reefs 26:71-78
Brown MB, Edwards MS, Kim KY (2014) Effects of climate change on the physiology of giant kelp, Macrocystis pyrifera, and grazing by purple urchin, Strongylocentrotus purpuratus Algae 29:203
Bruggemann J, Van Kessel A, Van Rooij J, Breeman A (1996) Bioerosion and sediment ingestion by the Caribbean parrotfish Scarus vetula and Sparisoma viride: implications of fish size, feeding mode and habitat use Marine Ecology Progress Series 134:59-71
Brundu G, Vian Monleón L, Vallainc D, Carboni S (2016) Effects of larval diet and metamorphosis cue on survival and growth of sea urchin post-larvae (Paracentrotus lividus; Lamarck, 1816) Aquaculture 465:265-271 doi:https://doi.org/10.1016/j.aquaculture.2016.09.014
Bulleri F, Benedetti-Cecchi L, Cinelli F (1999) Grazing by the sea urchins Arbacia lixula L. and Paracentrotus lividus Lam. in the Northwest Mediterranean Journal of Experimental Marine Biology and Ecology 241:81-95 doi:http://dx.doi.org/10.1016/S0022-0981(99)00073-8
Burke L, Reytar K, Spalding M, Perry A (2011) Reefs at risk revisited.
Burkepile DE, Hay ME (2006) HERBIVORE VS. NUTRIENT CONTROL OF MARINE PRIMARY PRODUCERS: CONTEXT-DEPENDENT EFFECTS Ecology 87:3128-3139
Burkepile DE, Hay ME (2008) Herbivore species richness and feeding complementarity affect community structure and function on a coral reef Proceedings of the National Academy of Sciences 105:16201-16206
Burkepile DE, Hay ME (2009) Nutrient versus herbivore control of macroalgal community development and coral growth on a Caribbean reef Marine Ecology Progress Series 389:71-84
Butler I, Sommer B, Zann M, Zhao J-x, Pandolfi J (2013) The impacts of flooding on the high-latitude, terrigenoclastic influenced coral reefs of Hervey Bay, Queensland, Australia Coral reefs 32:1149-1163
Byrne M (1990) Annual reproductive cycles of the commercial sea urchinParacentrotus lividus from an exposed intertidal and a sheltered subtidal habitat on the west coast of Ireland Marine Biology 104:275-289
Byrne M, Andrew N, Worthington D, Brett P (1998) Reproduction in the diadematoid sea urchin Centrostephanus rodgersii in contrasting habitats along the coast of New South Wales, Australia Marine Biology 132:305-318
Byrne M, Ho M, Selvakumaraswamy P, Nguyen HD, Symon AD, Davis AR (2009) Temperature, but Not pH, Compromises Sea Urchin Fertilization and Early Development under Near-Future Climate Change Scenarios Proceedings: Biological Sciences 276:1883-1888 doi:10.2307/30244023
Byrne M, Przeslawski R (2013) Multistressor impacts of warming and acidification of the ocean on marine invertebrates' life histories Integrative and Comparative Biology 53:582-596
Byrnes JE, Cardinale BJ, Reed DC (2013a) Interactions between sea urchin grazing and prey diversity on temperate rocky reef communities Ecology 94:1636-1646
Byrnes JE et al. (2013b) The sea urchin–the ultimate herbivore and biogeographic variability in its ability to deforest kelp ecosystems. PeerJ PrePrints,
Cabana G, Rasmussen JB (1996) Comparison of aquatic food chains using nitrogen isotopes Proceedings of the National Academy of Sciences 93:10844-10847
Cabanillas-Terán N, Loor-Andrade P, Rodríguez-Barreras R, Cortés J (2016) Trophic ecology of sea urchins in coral-rocky reef systems, Ecuador PeerJ 4:e1578
Carpenter R (1984) Predator and population density control of homing behavior in the Caribbean echinoid Diadema antillarum Marine Biology 82:101-108

Carpenter R (1990) Mass mortality ofDiadema antillarum Marine Biology 104:67-77
Carpenter RC (1988) Mass mortality of a Caribbean sea urchin: immediate effects on community metabolism and other herbivores Proceedings of the National Academy of Sciences 85:511-514
Carpenter RC, Edmunds PJ (2006) Local and regional scale recovery of Diadema promotes recruitment of scleractinian corals Ecology letters 9:271-280
Carreiro-Silva M, McClanahan T (2012) Macrobioerosion of dead branching Porites, 4 and 6 years after coral mass mortality Marine Ecology Progress Series 458:103-122
Carreiro-Silva M, McClanahan TR (2001) Echinoid bioerosion and herbivory on Kenyan coral reefs: the role of protection from fishing Journal of Experimental Marine Biology and Ecology 262:133-153 doi:http://dx.doi.org/10.1016/S0022-0981(01)00288-X
Catarino AI, De Ridder C, Gonzalez M, Gallardo P, Dubois P (2012) Sea urchin Arbacia dufresnei (Blainville 1825) larvae response to ocean acidification Polar biology 35:455-461
Chazottes V, Le Campion-Alsumard T, Peyrot-Clausade M, Cuet P (2002) The effects of eutrophication-related alterations to coral reef communities on agents and rates of bioerosion (Reunion Island, Indian Ocean) Coral reefs 21:375-390
Cheung WW, Lam VW, Sarmiento JL, Kearney K, Watson R, Pauly D (2009) Projecting global marine biodiversity impacts under climate change scenarios Fish and Fisheries 10:235-251
Cheung WW et al. (2012) Climate-change induced tropicalisation of marine communities in Western Australia Marine and Freshwater Research 63:415-427
Chong-Seng K, Nash K, Bellwood D, Graham N (2014) Macroalgal herbivory on recovering versus degrading coral reefs Coral Reefs 33:409-419
Clark D, Lamare M, Barker M (2009) Response of sea urchin pluteus larvae (Echinodermata: Echinoidea) to reduced seawater pH: a comparison among a tropical, temperate, and a polar species Marine Biology 156:1125-1137 doi:10.1007/s00227-009-1155-8
Cobb J, Lawrence JM (2005) Diets and coexistence of the sea urchins Lytechinus variegatus and Arbacia punctulata (Echinodermata) along the central Florida gulf coast Marine Ecology Progress Series 295:171-182
Connell SD, Irving AD (2008) Integrating ecology with biogeography using landscape characteristics: a case study of subtidal habitat across continental Australia Journal of Biogeography 35:1608-1621
Contreras S, Castilla J (1987) Feeding behavior and morphological adaptations in two sympatric sea urchin species in central Chile Marine Ecology Progress Series 38:217-224
Cook E, Kelly M (2007a) Enhanced production of the sea urchin Paracentrotus lividus in integrated open-water cultivation with Atlantic salmon Salmo salar Aquaculture 273:573-585
Cook EJ, Kelly MS (2007b) Effect of variation in the protein value of the red macroalga Palmaria palmata on the feeding, growth and gonad composition of the sea urchins Psammechinus miliaris and Paracentrotus lividus (Echinodermata) Aquaculture 270:207-217
Cook K, Vanderklift MA (2011) Depletion of predatory fish by fishing in a temperate reef ecosystem leads to indirect effects on prey, but not to lower trophic levels Marine Ecology Progress Series 432:195-205
Coppard S, Campbell A (2005a) Distribution and abundance of regular sea urchins on two coral reefs in Fiji MICRONESICA-AGANA- 37:249
Coppard SE, Campbell AC (2005b) Lunar periodicities of diadematid echinoids breeding in Fiji Coral Reefs 24:324-332
Cottee-Jones HEW, Whittaker RJ (2012) Perspective: the keystone species concept: a critical appraisal Frontiers of Biogeography 4
Cowen RK (1983) The effects of sheephead (Semicossyphus pulcher) predation on red sea urchin (Strongylocentrotus franciscanus) populations: an experimental analysis Oecologia 58:249-255

Coyer JA, Ambrose RF, Engle JM, Carroll JC (1993) Interactions between corals and algae on a temperate zone rocky reef: mediation by sea urchins Journal of Experimental Marine Biology and Ecology 167:21-37 doi:http://dx.doi.org/10.1016/0022-0981(93)90181-M
Cresswell GR, Golding T (1980) Observations of a south-flowing current in the southeastern Indian Ocean Deep Sea Research Part A Oceanographic Research Papers 27:449-466
Davidson TM, Grupe BM (2015) Habitat modification in tidepools by bioeroding sea urchins and implications for fine-scale community structure Marine Ecology 36:185-194
Davis AM, Blanchette ML, Pusey BJ, Jardine TD, Pearson RG (2012) Gut content and stable isotope analyses provide complementary understanding of ontogenetic dietary shifts and trophic relationships among fishes in a tropical river Freshwater Biology 57:2156-2172
De'ath G, Fabricius KE, Sweatman H, Puotinen M (2012) The 27–year decline of coral cover on the Great Barrier Reef and its causes Proceedings of the National Academy of Sciences 109:17995-17999 doi:10.1073/pnas.1208909109
Deagle BE, Gales NJ, Evans K, Jarman SN, Robinson S, Trebilco R, Hindell MA (2007) Studying Seabird Diet through Genetic Analysis of Faeces: A Case Study on Macaroni Penguins (<italic>Eudyptes chrysolophus</italic>) PLoS ONE 2:e831 doi:10.1371/journal.pone.0000831
Dee LE, Witman JD, Brandt M (2012) Refugia and top-down control of the pencil urchin Eucidaris galapagensis in the Galápagos Marine Reserve Journal of Experimental Marine Biology and Ecology 416:135-143 doi:http://dx.doi.org/10.1016/j.jembe.2012.02.016
Depczynski M et al. (2013) Bleaching, coral mortality and subsequent survivorship on a West Australian fringing reef Coral Reefs 32:233-238
Dix T (1977) Reproduction in Tasmanian populations of <I>Heliocidaris erythrogramma</I> (Echinodermata : Echinometridae) Marine and Freshwater Research 28:509-520 doi:http://dx.doi.org/10.1071/MF9770509
Dixson DL, Abrego D, Hay ME (2014) Chemically mediated behavior of recruiting corals and fishes: a tipping point that may limit reef recovery Science 345:892-897
Doney SC et al. (2011) Climate change impacts on marine ecosystems
Doropoulos C, Ward S, Diaz-Pulido G, Hoegh-Guldberg O, Mumby PJ (2012) Ocean acidification reduces coral recruitment by disrupting intimate larval-algal settlement interactions Ecology Letters 15:338-346
Drummond A (1991) Reproduction of the sea urchin Stomopneustes variolaris (Lam.) on the east coast of South Africa Invertebrate reproduction & development 20:259-265
Drummond A (1995) Reproduction of the sea urchins Echinometra mathaei and Diadema savignyi on the South African eastern coast Marine and freshwater research 46:751-755
Dumas J-P, Langlois TJ, Clarke KR, Waddington KI (2013) Strong preference for decapod prey by the western rock lobster Panulirus cygnus Journal of Experimental Marine Biology and Ecology 439:25-34
Dumont CP, Lau DC, Astudillo JC, Fong KF, Chak ST, Qiu J-W (2013) Coral bioerosion by the sea urchin Diadema setosum in Hong Kong: Susceptibility of different coral species Journal of Experimental Marine Biology and Ecology 441:71-79
Dupont S, Dorey N, Stumpp M, Melzner F, Thorndyke M (2013) Long-term and trans-life-cycle effects of exposure to ocean acidification in the green sea urchin Strongylocentrotus droebachiensis Marine Biology 160:1835-1843
Dworjanyn SA, Pirozzi I, Liu W (2007) The effect of the addition of algae feeding stimulants to artificial diets for the sea urchin Tripneustes gratilla Aquaculture 273:624-633
Eakin CM (1996) Where have all the carbonates gone? A model comparison of calcium carbonate budgets before and after the 1982–1983 El Nino at Uva Island in the eastern Pacific Coral Reefs 15:109-119 doi:10.1007/bf01771900

Ebert T (1980) Relative growth of sea urchin jaws: an example of plastic resource allocation Bulletin of Marine Science 30:467-474
Ebert TA, Hernández JC, Clemente S (2014) Annual reversible plasticity of feeding structures: cyclical changes of jaw allometry in a sea urchin Proceedings of the Royal Society of London B: Biological Sciences 281:20132284
Edgar G (1983) The ecology of south-east Tasmanian phytal animal communities. II. Seasonal change in plant and animal populations Journal of Experimental Marine Biology and Ecology 70:159-179
Edinger EN, Jompa J, Limmon GV, Widjatmoko W, Risk MJ (1998) Reef degradation and coral biodiversity in Indonesia: effects of land-based pollution, destructive fishing practices and changes over time Marine Pollution Bulletin 36:617-630
Edmunds PJ, Carpenter RC (2001) Recovery of Diadema antillarum reduces macroalgal cover and increases abundance of juvenile corals on a Caribbean reef Proceedings of the National Academy of Sciences 98:5067-5071
Elliott J (1972) Rates of gastric evacuation in brown trout, Salmo trutta L Freshwater Biology 2:1-18
Elliott J, Persson L (1978) The estimation of daily rates of food consumption for fish The Journal of Animal Ecology:977-991
Estes JA, Tinker M, Bodkin JL (2010) Using ecological function to develop recovery criteria for depleted species: sea otters and kelp forests in the Aleutian archipelago Conservation Biology 24:852-860
Fabricius KE (2005) Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis Marine pollution bulletin 50:125-146
Fabry VJ, Seibel BA, Feely RA, Orr JC (2008) Impacts of ocean acidification on marine fauna and ecosystem processes ICES Journal of Marine Science: Journal du Conseil 65:414-432
Feehan C, Scheibling RE, Lauzon-Guay J (2012) Aggregative feeding behavior in sea urchins leads to destructive grazing in a Nova Scotian kelp bed Marine Ecology Progress Series 444:69-83
Feehan CJ, Scheibling RE (2014) Effects of sea urchin disease on coastal marine ecosystems Marine biology 161:1467-1485
Feely RA, Sabine CL, Lee K, Berelson W, Kleypas J, Fabry VJ, Millero FJ (2004) Impact of anthropogenic CO2 on the CaCO3 system in the oceans Science 305:362-366
Fell FJ (1975) The echinoid genus Centrostephanus in the South Pacific Ocean with a description of a new species Journal of the Royal Society of New Zealand 5:179-193
Feng M, Waite A, Thompson P (2009a) Climate variability and ocean production in the Leeuwin Current system off the west coast of Western Australia Journal of the Royal Society of Western Australia 92:67-81
Feng M, Weller E, Hill K (2009b) The Leeuwin Current A marine climate change impacts and adaptation report card for Australia
Ferrari R, Gonzalez-Rivero M, Ortiz JC, Mumby PJ (2012) Interaction of herbivory and seasonality on the dynamics of Caribbean macroalgae Coral Reefs 31:683-692
Filbee-Dexter K, Scheibling RE (2014) Sea urchin barrens as alternative stable states of collapsed kelp ecosystems Marine ecology progress series 495:1-25
Flukes E, Johnson C, Ling S (2012) Forming sea urchin barrens from the inside out: an alternative pattern of overgrazing Marine Ecology Progress Series 464:179-194
Fodrie F, Heck KL, Powers SP, Graham WM, Robinson KL (2010) Climate-related, decadal-scale assemblage changes of seagrass-associated fishes in the northern Gulf of Mexico Global Change Biology 16:48-59
Foo SA, Dworjanyn SA, Poore AG, Byrne M (2012) Adaptive capacity of the habitat modifying sea urchin Centrostephanus rodgersii to ocean warming and ocean acidification: performance of early embryos PLoS One 7:e42497
Forcucci D (1994) Population density, recruitment and 1991 mortality event of Diadema antillarum in the Florida Keys Bulletin of Marine Science 54:917-928

Foster T, Short J, Falter J, Ross C, McCulloch M (2014) Reduced calcification in Western Australian corals during anomalously high summer water temperatures Journal of Experimental Marine Biology and Ecology 461:133-143
Freeman S (2003) Size-dependent distribution, abundance and diurnal rhythmicity patterns in the short-spined sea urchin Anthocidaris crassispina Estuarine, Coastal and Shelf Science 58:703-713
Freiwald J, Wisniewski CJ, Abbott D (2016) Northward range extension of the crowned sea urchin (Centrostephanus coronatus) to Monterey Bay, California California Fish and Game 102:37-40
Fry B (1991) Stable Isotope Diagrams of Freshwater Food Webs Ecology 72:2293-2297 doi:10.2307/1941580
Fry B (2006) Stable isotope ecology vol 521. Springer,
Fuji A (1962) Studies on the biology of the sea urchin: V. food consumption of Strongylocentrotus intermedius Japanese journal of ecology 12:181-186
Galloway A, Lowe A, Sosik E, Yeung J, Duggins D (2013) Fatty acid and stable isotope biomarkers suggest microbe-induced differences in benthic food webs between depths Limnol Oceanogr 58:1451-1462
Galloway AW, Britton-Simmons KH, Duggins DO, Gabrielson PW, Brett MT (2012) Fatty Acid Signatures Differentiate Marine Macrophytes At Ordinal and Family Ranks1 Journal of Phycology 48:956-965
Garrido C, Barber B (2001) Effects of temperature and food ration on gonad growth and oogenesis of the green sea urchin, Strongylocentrotus droebachiensis Marine Biology 138:447-456
Gaudette J, Wahle RA, Himmelman JH (2006) Spawning events in small and large populations of the green sea urchin Strongylocentrotus droebachiensis as recorded using fertilization assays Limnology and oceanography 51:1485-1496
Gherardi F (1991) Eco-ethological aspects of the symbiosis between the shrimp Athanas indicus (Coutière 1903) and the sea urchin Echinometra mathaei (de Blainville 1825) Tropical Zoology 4:107-128
Giese A, Krishnaswamy S, Vasu B, Lawrence J (1964) Reproductive and biochemical studies on a sea urchin, Stomopneustes variolaris, from Madras Harbor Comparative biochemistry and physiology 13:367-380
Giese A, Pearse J (1974) Reproducyion of Marine Invertebrates. New York Academic Press,
Giese A, Pearse J, Pearse V (1987) Reproduction of Marine Invertebrates vol 9. General aspects; Seeking unity in diversity. Boxwood press, Pacific Groove
Girard D, Clemente S, Toledo-Guedes K, Brito A, Hernández JC (2012) A mass mortality of subtropical intertidal populations of the sea urchin Paracentrotus lividus: analysis of potential links with environmental conditions Marine Ecology 33:377-385
Glynn PJ, Glynn PW, Riegl B (2017) El Niño, echinoid bioerosion and recovery potential of an isolated Galápagos coral reef: a modeling perspective Marine Biology 164:146 doi:10.1007/s00227-017-3175-0
Glynn PW (1988) El Nifio warming, coral mortality and reef framework destruction by echinoid bioerosion in the eastern Pacific Galaxea 7:129-160
Glynn PW, Manzello DP (2015) Bioerosion and coral reef growth: a dynamic balance. In: Coral reefs in the Anthropocene. Springer, pp 67-97
Glynn PW, Wellington GM, Birkeland C (1979) Coral reef growth in the Galapagos: limitation by sea urchins Science 203:47-49
Godfrey J, Ridgway K (1985) The large-scale environment of the poleward-flowing Leeuwin Current, Western Australia: longshore steric height gradients, wind stresses and geostrophic flow Journal of Physical Oceanography 15:481-495
Golubic S, Perkins RD, Lukas KJ (1975) Boring microorganisms and microborings in carbonate substrates. In: The study of trace fossils. Springer, pp 229-259

Gonor JJ (1973) Reproductive cycles in oregon populations of the echlnoid, Strongylocentrotvs purpuratus (Stimpson). I. Annual gonad growth and ovarian gametogenic cycles Journal of Experimental Marine Biology and Ecology 12:45-64
González-Irusta J, De Cerio FG, Canteras J (2010) Reproductive cycle of the sea urchin Paracentrotus lividus in the Cantabrian Sea (northern Spain): environmental effects Journal of the Marine Biological Association of the United Kingdom 90:699-709
Graham N, Nash K (2013) The importance of structural complexity in coral reef ecosystems Coral Reefs 32:315-326
Grant A, Tyler P (1983) The analysis of data in studies of invertebrate reproduction. I. Introduction and statistical analysis of gonad indices and maturity indices International Journal of Invertebrate Reproduction 6:259-269
Grey J, Jones RI, Sleep D (2001) Seasonal changes in the importance of the source of organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope analysis Limnology and Oceanography 46:505-513
Griffin S, Garcia R, Weil E (2003) Bioerosion in coral reef communities in southwest Puerto Rico by the sea urchin Echinometra viridis Marine Biology 143:79-84
Guest M, Frusher S, Nichols P, Johnson C, Wheatley K (2009) Trophic effects of fishing southern rock lobster Jasus edwardsii shown by combined fatty acid and stable isotope analyses Marine ecology progress series 388:169-184
Guidetti P (2006) Estimating body size of sea urchins, Paracentrotus lividus and Arbacia lixula, from stomach contents of Diplodus sargus, a Mediterranean predatory fish Journal of Applied Ichthyology 22:91-93
Hagen NT (2008) Enlarged lantern size in similar-sized, sympatric, sibling species of Strongylocentrotid sea urchins: from phenotypic accommodation to functional adaptation for durophagy Marine Biology 153:907-924
Hagen NT, Mann K (1994) Experimental analysis of factors influencing the aggregating behaviour of the green sea urchin Strongylocentrotus droebachiensis (Müller) Journal of experimental marine biology and ecology 176:107-126
Hallock P (1988) The role of nutrient availability in bioerosion: consequences to carbonate buildups Palaeogeography, Palaeoclimatology, Palaeoecology 63:275-291
Handeland SO, Imsland AK, Stefansson SO (2008) The effect of temperature and fish size on growth, feed intake, food conversion efficiency and stomach evacuation rate of Atlantic salmon post-smolts Aquaculture 283:36-42
Hardy CM, Krull ES, Hartley DM, Oliver RL (2010) Carbon source accounting for fish using combined DNA and stable isotope analyses in a regulated lowland river weir pool Mol Ecol 19:197-212 doi:10.1111/j.1365-294X.2009.04411.x
Harrold C, Pearse JS (1987) The ecological role of echinoderms in kelp forests Echinoderm studies 2:137-233
Harrold C, Reed DC (1985) Food availability, sea urchin grazing, and kelp forest community structure Ecology:1160-1169
Hart M, Scheibling RE (1988) Heat waves, baby booms, and the destruction of kelp beds by sea urchins Marine Biology 99:167-176
Hatcher B, Rimmer D The role of grazing in controlling benthic community structure on a high latitude coral reef: measurements of grazing intensity. In: Proc. 5th Int. Coral Reef Congr. Tahiti, 1985. pp 291-297
Heithaus MR, Frid A, Wirsing AJ, Worm B (2008) Predicting ecological consequences of marine top predator declines Trends in Ecology & Evolution 23:202-210
Hendriks IE, Duarte CM, Álvarez M (2010) Vulnerability of marine biodiversity to ocean acidification: a meta-analysis Estuarine, Coastal and Shelf Science 86:157-164
Hereu B, Zabala M, Linares C, Sala E (2004) Temporal and spatial variability in settlement of the sea urchin Paracentrotus lividus in the NW Mediterranean Marine Biology 144:1011-1018

Hernández J, Clemente S, Brito A (2011) Effects of seasonality on the reproductive cycle of Diadema aff. antillarum in two contrasting habitats: implications for the establishment of a sea urchin fishery Marine Biology 158:2603
Hernández J, Russell M (2010) Substratum cavities affect growth-plasticity, allometry, movement and feeding rates in the sea urchin Strongylocentrotus purpuratus Journal of Experimental Biology 213:520-525
Hernández JC, Brito A, García N, Gil-Rodríguez MC, Herrera G, Cruz-Reyes A, Falcón JM (2006) Spatial and seasonal variation of the gonad index of Diadema antillarum (Echinodermata: Echinoidea) in the Canary Islands Scientia Marina 70:689-698
Hernández JC, Clemente S, Girard D, Pérez-Ruzafa Á, Brito A (2010) Effect of temperature on settlement and postsettlement survival in a barrens-forming sea urchin Marine Ecology Progress Series 413:69-80
Hernández JC, Clemente S, Sangil C, Brito A (2008) The key role of the sea urchin Diadema aff. antillarum in controlling macroalgae assemblages throughout the Canary Islands (eastern subtropical Atlantic): an spatio-temporal approach Marine Environmental Research 66:259-270
Herrera-Escalante T, López-Pérez R, Leyte-Morales G (2005) Bioerosion caused by the sea urchin Diadema mexicanum (Echinodermata: Echinoidea) at Bahías de Huatulco, Western Mexico Revista de biología tropical 53:263
Highsmith RC (1981) Coral bioerosion: damage relative to skeletal density The American Naturalist 117:193-198
Hill NA, Blount C, Poore AG, Worthington D, Steinberg PD (2003) Grazing effects of the sea urchin Centrostephanus rodgersii in two contrasting rocky reef habitats: effects of urchin density and its implications for the fishery Marine and freshwater research 54:691-700
Hill SK, Lawrence JM (2003) Habitats and Characteristics of the Sea Urchins Lytechinus variegatus and Arbacia punctulata (Echinodermata) on the Florida Gulf-Coast Shelf Marine Ecology 24:15-30
Himmelman J, Steele D (1971) Foods and predators of the green sea urchin Strongylocentrotus droebachiensis in Newfoundland waters Marine Biology 9:315-322
Himmelman JH (1978) Reproductive cycle of the green sea urchin, Strongylocentrotus droebachiensis Canadian Journal of Zoology 56:1828-1836 doi:10.1139/z78-249
Himmelman JH (1986) Population biology of green sea urchins on rocky barrens Mar Ecol Prog Ser 33:295-306
Hixon MA, Brostoff WN (1983) Damselfish as keystone species in reverse: intermediate disturbance and diversity of reef algae Science(Washington) 220:511-513
Hoegh-Guldberg O, Bruno JF (2010) The impact of climate change on the world's marine ecosystems Science 328:1523-1528
Holmes KE (2000) Effects of eutrophication on bioeroding sponge communities with the description of new West Indian sponges, Cliona spp.(Porifera: Hadromerida: Clionidae) Invertebrate Biology 119:125-138
Holmes KE, Edinger EN, Limmon GV, Risk MJ (2000) Bioerosion of live massive corals and branching coral rubble on Indonesian coral reefs Marine Pollution Bulletin 40:606-617
Hönisch B et al. (2012) The geological record of ocean acidification science 335:1058-1063
Hori R, Phang VP, Lam TJ (1987) Preliminary study on the pattern of gonadal development of the sea urchin, Diadema setosum, off the coast of Singapore Zoological science 4:p665-673
Huggett MJ, King CK, Williamson JE, Steinberg PD (2005) Larval development and metamorphosis of the Australian diadematid sea urchin Centrostephanus rodgersii Invertebrate reproduction & development 47:197-204
Hughes T, Connell J (1999) Multiple stressors on coral reefs: a long-term perspective Limnology and oceanography 44:932-940

Hughes TP (1994) Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef Science-AAAS-Weekly Paper Edition 265:1547-1551
Hughes TP, Reed DC, Boyle M-J (1987) Herbivory on coral reefs: community structure following mass mortalities of sea urchins Journal of Experimental Marine Biology and Ecology 113:39-59
Humphries A, McClanahan T, McQuaid C (2014) Differential impacts of coral reef herbivores on algal succession in Kenya Marine Ecology Progress Series 504:119-132
Hussey NE, Dudley SF, McCarthy ID, Cliff G, Fisk AT (2011) Stable isotope profiles of large marine predators: viable indicators of trophic position, diet, and movement in sharks? Canadian Journal of Fisheries and Aquatic Sciences 68:2029-2045
Hutchings P (1986) Biological destruction of coral reefs Coral reefs 4:239-252
Hyslop EJ (1980) Stomach contents analysis—a review of methods and their application Journal of Fish Biology 17:411-429 doi:10.1111/j.1095-8649.1980.tb02775.x
Idjadi JA, Haring RN, Precht WF (2010) Recovery of the sea urchin Diadema antillarum promotes scleractinian coral growth and survivorship on shallow Jamaican reefs Marine Ecology Progress Series 403:91-100
Iliffe TM, Pearse JS (1982) Annual and lunar reproductive rhythms of the sea urchin, Diadema antillarum (Philippi) in Bermuda International Journal of Invertebrate Reproduction 5:139-148
Ishimatsu A, Kikkawa T, Hayashi M, Lee K-S, Kita J (2004) Effects of CO2 on Marine Fish: Larvae and Adults Journal of Oceanography 60:731-741 doi:10.1007/s10872-004-5765-y
Iverson SJ, Field C, Don Bowen W, Blanchard W (2004) Quantitative fatty acid signature analysis: a new method of estimating predator diets Ecological Monographs 74:211-235
Jacob U, Terpstra S, Brey T (2003) High-Antarctic regular sea urchins–the role of depth and feeding in niche separation Polar Biology 26:99-104
Jacquin A-G, Donval A, Guillou J, Leyzour S, Deslandes E, Guillou M (2006) The reproductive response of the sea urchins Paracentrotus lividus (G.) and Psammechinus miliaris (L.) to a hyperproteinated macrophytic diet Journal of experimental marine biology and ecology 339:43-54
James PJ, Heath PL (2008) The effects of season, temperature and photoperiod on the gonad development of Evechinus chloroticus Aquaculture 285:67-77
Jensen H, Kiljunen M, Amundsen PA (2012) Dietary ontogeny and niche shift to piscivory in lacustrine brown trout Salmo trutta revealed by stomach content and stable isotope analyses Journal of fish biology 80:2448-2462
Johansson C, Bellwood D, Depczynski M, Hoey A (2013) The distribution of the sea urchin Echinometra mathaei (de Blainville) and its predators on Ningaloo Reef, Western Australia: the implications for top-down control in an intact reef system Journal of Experimental Marine Biology and Ecology 442:39-46
Johnson C, Mann K Adaptations of Strongylocentrotus droebachiensis for survival on barren grounds in Nova Scotia. In: Echinoderms: Proceedings of the International Conference, 1982. JM Lawrence, Balkema, Rotterdam, pp 277-283
Johnson C, Valentine J, Pederson H A most unusual barrens: complex interactions between lobsters, sea urchins and algae facilitates spread of an exotic kelp in eastern Tasmania. In: Echinoderms: Munchen: Proceedings of the 11th International Echinoderm Conference, 6-10 October 2003, Munich, Germany, 2004. CRC Press, p 213
Johnson CR et al. (2011) Climate change cascades: Shifts in oceanography, species' ranges and subtidal marine community dynamics in eastern Tasmania Journal of Experimental Marine Biology and Ecology 400:17-32 doi:http://dx.doi.org/10.1016/j.jembe.2011.02.032
Johnson CR, Ling S, Ross D, Shepherd S, Miller K (2005) Establishment of the long-spined sea urchin (Centrostephanus rodgersii) in Tasmania: first assessment of potential threats to fisheries

Johnson MD, Carpenter RC (2012) Ocean acidification and warming decrease calcification in the crustose coralline alga Hydrolithon onkodes and increase susceptibility to grazing Journal of Experimental Marine Biology and Ecology 434:94-101
Jompa J, McCook LJ (2003) Contrasting effects of turf algae on corals

massive Porites spp. are unaffected by mixed-species turfs, but killed by the red alga Anotrichium tenue Marine Ecology Progress Series 258:79-86
Jong-Westman Md, March B, Carefoot T (1995) The effect of different nutrient formulations in artificial diets on gonad growth in the sea urchin Strongylocentrotus droebachiensis Canadian Journal of Zoology 73:1495-1502
Kailola PJ (1993) Australian fisheries resources. Bureau of Resource Sciences, Dept. of Primary Industries and Energy; Fisheries Research and Development Corp.,
Kawamata S (1997) Modelling the feeding rate of the sea urchin Strongylocentrotus nudus (A. Agassiz) on kelp Journal of Experimental Marine Biology and Ecology 210:107-127 doi:http://dx.doi.org/10.1016/S0022-0981(96)02707-4
Kawamata S (1998) Effect of wave-induced oscillatory flow on grazing by a subtidal sea urchin Strongylocentrotus nudus (A. Agassiz) Journal of Experimental Marine Biology and Ecology 224:31-48
Keats D, Steele D, South G (1984) Depth-dependent reproductive output of the green sea urchin, Strongylocentrotus droebachiensis (OF Müller), in relation to the nature and availability of food Journal of Experimental Marine Biology and Ecology 80:77-91
Keesing JK, Hall KC (1998) Review of harvests and status of world sea urchin fisheries points to opportunities for aquaculture
Kelly JR, Krumhansl KA, Scheibling RE (2012) Drift algal subsidies to sea urchins in low-productivity habitats Marine Ecology Progress Series 452:145-157
Kelly MS (2001) Environmental parameters controlling gametogenesis in the echinoid Psammechinus miliaris Journal of Experimental Marine Biology and Ecology 266:67-80
Kendrick GA, Lavery PS, Phillips JC Influence of Ecklonia radiata kelp canopy on structure of macro-algal assemblages in Marmion Lagoon, Western Australia. In: Sixteenth International Seaweed Symposium, 1999. Springer, pp 275-283
Kendrick GA, Walker DI (1994) ROLE OF RECRUITMENT IN STRUCTURING BEDS OF SARGASSUM SPP. (PHAEOPHYTA) AT ROTTNEST ISLAND, WESTERN AUSTRALIA1 Journal of Phycology 30:200-208 doi:10.1111/j.0022-3646.1994.00200.x
Kennedy B, Pearse JS (1975) Lunar synchronization of the monthly reproductive rhythm in the sea urchin Centrostephanus coronatus Verrill Journal of Experimental Marine Biology and Ecology 17:323-331
Kennelly SJ (1987) Inhibition of kelp recruitment by turfing algae and consequences for an Australian kelp community Journal of Experimental Marine Biology and Ecology 112:49-60
Keough JR, Sierszen ME, Hagley CA (1996) Analysis of a Lake Superior coastal food web with stable isotope techniques Limnology and Oceanography 41:136-146
Kharlamenko V, Kiyashko S, Imbs A, Vyshkvartzev D (2001) Identification of food sources of invertebrates from the seagrass Zostera marina community using carbon and sulfur stable isotope ratio and fatty acid analyses Marine ecology Progress series 220:103-117
Killian CE et al. (2011) Self-Sharpening Mechanism of the Sea Urchin Tooth Advanced Functional Materials 21:682-690
King C, Hoegh-Guldberg O, Byrne M (1994) Reproductive cycle of Centrostephanus rodgersii (Echinoidea), with recommendations for the establishment of a sea urchin fishery in New South Wales Marine Biology 120:95-106
Kintzing MD, Butler IV MJ (2014) Effects of predation upon the long-spined sea urchin Diadema antillarum by the spotted spiny lobster Panulirus guttatus Marine Ecology Progress Series 495:185-191

Kleypas JA, McManus JW, Meñez LA (1999) Environmental limits to coral reef development: where do we draw the line? American Zoologist:146-159
Klinger TS, Lawrence JM (1985) Distance perception of food and the effect of food quantity on feeding behavior of Lytechinus variegatus (Lamarck)(Echinodermata: Echinoidea) Marine & Freshwater Behaviour & Phy 11:327-344
Klumpp DW, Salita-Espinosa J, Fortes M (1993) Feeding ecology and trophic role of sea urchins in a tropical seagrass community Aquatic Botany 45:205-229
Kolts JM, Lovvorn JR, North CA, Grebmeier JM, Cooper LW (2013) Relative value of stomach contents, stable isotopes, and fatty acids as diet indicators for a dominant invertebrate predator (Chionoecetes opilio) in the northern Bering Sea Journal of Experimental Marine Biology and Ecology 449:274-283
Konar B (2000) Seasonal inhibitory effects of marine plants on sea urchins: structuring communities the algal way Oecologia 125:208-217
Kroeker KJ et al. (2013) Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming Global change biology 19:1884-1896
Kurihara H, Yin R, Nishihara GN, Soyano K, Ishimatsu A (2013) Effect of ocean acidification on growth, gonad development and physiology of the sea urchin Hemicentrotus pulcherrimus Aquatic Biology 18:281-292
Lafferty KD (2004) Fishing for lobsters indirectly increases epidemics in sea urchins Ecological Applications 14:1566-1573
Lamare M, Stewart B (1998) Mass spawning by the sea urchin Evechinus chloroticus (Echinodermata: Echinoidea) in a New Zealand fiord Marine Biology 132:135-140
Lamare MD, Brewin PE, Barker MF, Wing SR (2002) Reproduction of the sea urchin Evechinus chloroticus (Echinodermata: Echinoidea) in a New Zealand fiord New Zealand Journal of Marine and Freshwater Research 36:719-732
Langdon M (2012) The ecology of the grazing urchin Echinometra mathaei at Ningaloo Marine Park. Murdoch University
Langdon MW, Paling EI, Van Keulen M (2011) The development of urchin barrens in seagrass meadows at Luscombe Bay, Western Australia from 1985 to 2004 Pacific Conservation Biology 17:48
Lau DC, Lau SC, Qian P-Y, Qiu J-W (2009) Morphological plasticity and resource allocation in response to food limitation and hyposalinity in a sea urchin Journal of Shellfish Research 28:383-388
Lauzon-Guay J-S, Scheibling RE, Barbeau MA (2008) Modelling phase shifts in a rocky subtidal ecosystem Marine Ecology progress Series
Lawrence JM (1975) On the relationships between marine plants and sea urchins
Lawrence JM (2013) Sea urchins: biology and ecology vol 38. Academic Press,
Layman CA et al. (2012) Applying stable isotopes to examine food-web structure: an overview of analytical tools Biological Reviews 87:545-562
Leclercq NIc, Gattuso JEANP, Jaubert JEAN (2000) CO2 partial pressure controls the calcification rate of a coral community Global Change Biology 6:329-334 doi:10.1046/j.1365-2486.2000.00315.x
Leleu K, Remy-Zephir B, Grace R, Costello MJ (2012) Mapping habitats in a marine reserve showed how a 30-year trophic cascade altered ecosystem structure Biological Conservation 155:193-201
Lemoine NP, Burkepile DE (2012) Temperature-induced mismatches between consumption and metabolism reduce consumer fitness Ecology 93:2483-2489
Lepoint G, Dauby P, Gobert S (2004) Applications of C and N stable isotopes to ecological and environmental studies in seagrass ecosystems Marine pollution bulletin 49:887-891
Lessios H (1981) Reproductive periodicity of the echinoids Diadema and Echinometra on the two coasts of Panama Journal of Experimental Marine Biology and Ecology 50:47-61
Lessios H (1988a) Mass mortality of Diadema antillarum in the Caribbean: what have we learned? Annual Review of Ecology and Systematics 19:371-393

Lessios H (1988b) Population dynamics of Diadema antillarum (Echinodermata: Echinoidea) following mass mortality in Panama Marine Biology 99:515-526
Lessios H (1995) Diadema antillarum 10 years after mass mortality: still rare, despite help from a competitor Proceedings of the Royal Society of London B: Biological Sciences 259:331-337
Lessios H, Cubit J, Robertson D, Shulman M, Parker M, Garrity S, Levings S (1984a) Mass mortality of Diadema antillarum on the Caribbean coast of Panama Coral reefs 3:173-182
Lessios HA (2013) Natural population density fluctuations of echinoids. Do they help predict the future Climate change perspectives from the Atlantic: past, present and future Servicios de Publicaciones, Universidad de La Laguna:341-359
Lessios HA, Cubit JD, Robertson DR, Shulman MJ, Parker MR, Garrity SD, Levings SC (1984b) Mass mortality of Diadema antillarum on the Caribbean coast of Panama Coral Reefs 3:173-182
Lessios HA, Garrido MJ, Kessing BD (2001) Demographic history of Diadema antillarum, a keystone herbivore on Caribbean reefs Proceedings of the Royal Society of London B: Biological Sciences 268:2347-2353
Levitan D (1991) Skeletal changes in the test and jaws of the sea urchinDiadema antillarum in response to food limitation Marine Biology 111:431-435
Levitan DR (1989) Density-Dependent Size Regulation in Diadema Antillarum: Effects on Fecundity and Survivorship Ecology 70:1414-1424
Levitan DR, Sewell MA, Chia F-S (1992) How distribution and abundance influence fertilization success in the sea urchin Strongylocentotus franciscanus Ecology 73:248-254
Lewis JB (1964) Feeding and digestion in the tropical sea urchin Diadema antillarum Philippi Canadian Journal of Zoology 42:549-557
Ling S, Ibbott S, Sanderson J (2010) Recovery of canopy-forming macroalgae following removal of the enigmatic grazing sea urchin Heliocidaris erythrogramma Journal of Experimental Marine Biology and Ecology 395:135-146
Ling S, Johnson C (2009) Population dynamics of an ecologically important range-extender: kelp beds versus sea urchin barrens Marine Ecology Progress Series 374:113-125
Ling S, Johnson C, Frusher S, Ridgway K (2009a) Overfishing reduces resilience of kelp beds to climate-driven catastrophic phase shift Proceedings of the National Academy of Sciences 106:22341-22345
Ling S et al. (2015) Global regime shift dynamics of catastrophic sea urchin overgrazing Phil Trans R Soc B 370:20130269
Ling SD (2008) Range Expansion of a Habitat-Modifying Species Leads to Loss of Taxonomic Diversity: A New and Impoverished Reef State Oecologia 156:883-894 doi:10.2307/40309577
Ling SD, Johnson C, Frusher S, King C (2008) Reproductive potential of a marine ecosystem engineer at the edge of a newly expanded range Global Change Biology 14:907-915
Ling SD, Johnson CR, Ridgway K, Hobday AJ, Haddon M (2009b) Climate-driven range extension of a sea urchin: inferring future trends by analysis of recent population dynamics Global Change Biology 15:719-731 doi:10.1111/j.1365-2486.2008.01734.x
Lissner AL (1980) Some effects of turbulence on the activity of the sea urchin Centrostephanus coronatus Verrill Journal of Experimental Marine Biology and Ecology 48:185-193
Livore JP, Connell SD (2012a) Effects of food origin and availability on sea urchin condition and feeding behaviour Journal of sea research 68:1-5
Livore JP, Connell SD (2012b) Reducing per capita food supply alters urchin condition and habitat Marine biology 159:967-973
Lowry ML, Pearse JS (1973) Abalones and sea urchins in an area inhabited by sea otters Marine Biology 23:213-219

Lubchenco J, Gaines SD (1981) A Unified Approach to Marine Plant-Herbivore Interactions. I. Populations and Communities Annual Review of Ecology and Systematics 12:405-437 doi:10.2307/2097118
Lyimo TJ, Mamboya F, Hamisi M, Lugomela C (2011) Food preference of the sea urchin Tripneustes gratilla (Linnaeus, 1758) in tropical seagrass habitats at Dar es Salaam, Tanzania Journal of Ecology and the Natural Environment 3:415-423
Ma Y et al. (2009) The grinding tip of the sea urchin tooth exhibits exquisite control over calcite crystal orientation and Mg distribution Proceedings of the National Academy of Sciences 106:6048-6053
Ma Y, Cohen SR, Addadi L, Weiner S (2008) Sea Urchin Tooth Design: An "All-Calcite" Polycrystalline Reinforced Fiber Composite for Grinding Rocks Advanced Materials 20:1555-1559
Maier C, Hegeman J, Weinbauer MG, Gattuso J-P (2009) Calcification of the cold-water coral Lophelia pertusa under ambient and reduced pH Biogeosciences Discussions
Mamelona J, Pelletier É (2005) Green urchin as a significant source of fecal particulate organic matter within nearshore benthic ecosystems Journal of Experimental Marine Biology and Ecology 314:163-174 doi:http://dx.doi.org/10.1016/j.jembe.2004.08.026
Mann KH, Wright JL, Welsford BE, Hatfield E (1984) Responses of the sea urchin Strongylocentrotusdroebachiensis (OF Müller) to water-borne stimuli from potential predators and potential food algae Journal of Experimental Marine Biology and Ecology 79:233-244
Manullang C, Tsuchiya M, Ambariyanto A, Permata D (2014) Impact Test Size and Type of Echinometra mathaei as Agent of Bioerosion on Reef Flat (Pengaruh Ukuran dan Tipe Echinometra mathaei pada Bioerosi Karang) ILMU KELAUTAN: Indonesian Journal of Marine Sciences 19:75-80
Manzello DP et al. (2014) Galápagos coral reef persistence after ENSO warming across an acidification gradient Geophysical Research Letters 41:9001-9008
Mapstone B, Andrew N, Chancerelle Y, Salvat B (2007) Mediating effects of sea urchins on interactions among corals, algae and herbivorous fish in the Moorea lagoon, French Polynesia Marine Ecology Progress Series 332:143-153
Mayfield S, Branch GM (2000) Interrelations among rock lobsters, sea urchins, and juvenile abalone: implications for community management Canadian Journal of Fisheries and Aquatic Sciences 57:2175-2185
McBride SC, Price RJ, Tom PD, Lawrence JM, Lawrence AL (2004) Comparison of gonad quality factors: color, hardness and resilience, of Strongylocentrotus franciscanus between sea urchins fed prepared feed or algal diets and sea urchins harvested from the Northern California fishery Aquaculture 233:405-422
McClanahan T Effects of fishing and reef structure on East African coral reefs. In: Proceedings of the 8th international coral reef symposium, 1997. Smithsonian Tropical Research Institute, Balboa, Republic of Panama, pp 1533-1538
McClanahan T (1998) Predation and the distribution and abundance of tropical sea urchin populations Journal of Experimental Marine Biology and Ecology 221:231-255
McClanahan T (1999a) Predation and the control of the sea urchin Echinometra viridis and fleshy algae in the patch reefs of Glovers Reef, Belize Ecosystems 2:511-523
McClanahan T (1999b) Predation and the Control of the Sea Urchin Echinometra viridisand Fleshy Algae in the Patch Reefs of Glovers Reef, Belize Ecosystems 2:511-523
McClanahan T (2000) Recovery of a coral reef keystone predator,< i> Balistapus undulatus</i>, in East African marine parks Biological Conservation 94:191-198
McClanahan T, Kurtis J (1991) Population regulation of the rock-boring sea urchin< i> Echinometra mathaei</i>(de Blainville) Journal of Experimental Marine Biology and Ecology 147:121-146
McClanahan TR, Kamukuru AT, Muthiga NA, Yebio MG, Obura D (1996) Effect of Sea Urchin Reductions on Algae, Coral, and Fish Populations

Efectos de la reducción en la abundancia de erizos marinos sobre las poblaciones de algas, corales y peces Conservation Biology 10:136-154 doi:10.1046/j.1523-1739.1996.10010136.x
McClanahan TR, Nugues M, Mwachireya S (1994) Fish and sea urchin herbivory and competition in Kenyan coral reef lagoons: the role of reef management Journal of Experimental Marine Biology and Ecology 184:237-254 doi:http://dx.doi.org/10.1016/0022-0981(94)90007-8
McCook L, Jompa J, Diaz-Pulido G (2001) Competition between corals and algae on coral reefs: a review of evidence and mechanisms Coral Reefs 19:400-417 doi:10.1007/s003380000129
McCook LJ (1999) Macroalgae, nutrients and phase shifts on coral reefs: scientific issues and management consequences for the Great Barrier Reef Coral Reefs 18:357-367 doi:10.1007/s003380050213
McManus JW, Meñez LA, Kesner-Reyes KN, Vergara SG, Ablan M (2000) Coral reef fishing and coral-algal phase shifts: implications for global reef status ICES Journal of Marine Science: Journal du Conseil 57:572-578
McManus JW, Polsenberg JF (2004) Coral–algal phase shifts on coral reefs: ecological and environmental aspects Progress in Oceanography 60:263-279
McShane PE, Gerring PK, Anderson OA, Stewart RA (1996) Population differences in the reproductive biology of Evechinus chloroticus (Echinoidea: Echinometridae) New Zealand Journal of Marine and Freshwater Research 30:333-339 doi:10.1080/00288330.1996.9516720
Meidel S, Scheibling RE (1998) Annual reproductive cycle of the green sea urchin, Strongylocentrotus droebachiensis, in differing habitats in Nova Scotia, Canada Marine Biology 131:461-478
Meidel S, Scheibling RE (1999) Effects of food type and ration on reproductive maturation and growth of the sea urchin Strongylocentrotus droebachiensis Marine Biology 134:155-166
Melzner F et al. (2013) Future ocean acidification will be amplified by hypoxia in coastal habitats Marine Biology 160:1875-1888
Mercado-Molina AE et al. (2015) Revisiting the population status of the sea urchin Diadema antillarum in northern Puerto Rico Journal of the Marine Biological Association of the United Kingdom 95:1017-1024
Michael PJ, Hyndes GA, Vanderklift MA, Vergés A (2013) Identity and behaviour of herbivorous fish influence large-scale spatial patterns of macroalgal herbivory in a coral reef Marine Ecology Progress Series 482:227-240
Michaelidis B, Ouzounis C, Paleras A, PÃ¶rtner HO (2005) Effects of long-term moderate hypercapnia on acid–base balance and growth rate in marine mussels Mytilus galloprovincialis Marine Ecology Progress Series 293:109-118 doi:10.3354/meps293109
Miller R, Colodey A (1983) Widespread mass mortalities of the green sea urchin in Nova Scotia, Canada Marine Biology 73:263-267
Mills LS, Soulé ME, Doak DF (1993) The keystone-species concept in ecology and conservation BioScience 43:219-224
Mills SC, Peyrot-Clausade M, France Fontaine M (2000) Ingestion and transformation of algal turf by Echinometra mathaei on Tiahura fringing reef (French Polynesia) Journal of Experimental Marine Biology and Ecology 254:71-84
Minor M, Scheibling RE (1997) Effects of food ration and feeding regime on growth and reproduction of the sea urchin Strongylocentrotus droebachiensis Marine Biology 129:159-167
Mokady O, Lazar B, Loya Y (1996) Echinoid bioerosion as a major structuring force of Red Sea coral reefs The Biological Bulletin 190:367-372

Molinet C et al. (2012) Reproduction of the sea urchin Loxechinus albus across a bathymetric gradient in the Chilean Inland Sea Revista de biología marina y oceanografía 47:257-272
Moore C et al. (2013) Processes and patterns of oceanic nutrient limitation Nature Geoscience 6:701-710
Moore HB, Jutare T, Bauer J, Jones J (1963) The biology of Lytechinus variegatus Bulletin of Marine Science 13:23-53
Moore JA et al. (2012a) Unprecedented mass bleaching and loss of coral across 12 of latitude in Western Australia in 2010–11 PLoS One 7:e51807
Moore JAY et al. (2012b) Unprecedented Mass Bleaching and Loss of Coral across 12° of Latitude in Western Australia in 2010-11 PLoS ONE 7
Moore JK, Doney SC, Glover DM, Fung IY (2001) Iron cycling and nutrient-limitation patterns in surface waters of the World Ocean Deep Sea Research Part II: Topical Studies in Oceanography 49:463-507 doi:https://doi.org/10.1016/S0967-0645(01)00109-6
Morrison D (1988) Comparing Fish and Urchin Grazing in Shallow and Deeper Coral Reef Algal Communities Ecology 69:1367-1382 doi:10.2307/1941634
Moyer RP, Riegl B, Banks K, Dodge RE (2003) Spatial patterns and ecology of benthic communities on a high-latitude South Florida (Broward County, USA) reef system Coral Reefs 22:447-464
Mumby PJ (2006) The impact of exploiting grazers (Scaridae) on the dynamics of Caribbean coral reefs Ecological Applications 16:747-769
Murillo-Navarro R, Jiménez-Guirado D (2012) Relationships between algal food and gut and gonad conditions in the Mediterranean sea urchin Paracentrotus lividus (Lam.) Mediterranean Marine Science 13:227-238
Muthiga N (2003a) Coexistence and reproductive isolation of the sympatric echinoids Diadema savignyi Michelin and Diadema setosum (Leske) on Kenyan coral reefs Marine Biology 143:669-677
Muthiga NA (2003b) Coexistence and reproductive isolation of the sympatric echinoids Diadema savignyi Michelin and Diadema setosum (Leske) on Kenyan coral reefs Marine Biology 143:669-677 doi:10.1007/s00227-003-1095-7
Myhre S, Acevedo-Gutiérrez A (2007) Recovery of sea urchin Diadema antillarum populations is correlated to increased coral and reduced macroalgal cover Marine Ecology Progress Series 329:205-210
Norderhaug KM, Christie HC, Pedersen MF, Fredriksen S (2014) Predators of the destructive sea urchin grazer Strongylocentrotus droebachiensis on the Norwegian coast
Nyström M, Folke C, Moberg F (2000) Coral reef disturbance and resilience in a human-dominated environment Trends in Ecology & Evolution 15:413-417
O'Leary JK, McClanahan TR (2010) Trophic cascades result in large-scale coralline algae loss through differential grazer effects Ecology 91:3584-3597 doi:10.1890/09-2059.1
O'Leary J, Potts D, Braga J, McClanahan T (2012) Indirect consequences of fishing: reduction of coralline algae suppresses juvenile coral abundance Coral Reefs 31:547-559
Paine RT (1969) A note on trophic complexity and community stability The American Naturalist 103:91-93
Paine RT, Vadas RL (1969) The effects of grazing by sea urchins, Strongylocentrotus spp., on benthic algal populations Limnology and Oceanography 14:710-719
Palacios MdM, Zapata FA (2014) Fish community structure on coral habitats with contrasting architecture in the Tropical Eastern Pacific Revista de Biología Tropical 62:343-357
Pane EF, Barry JP (2007) Extracellular acid–base regulation during short-term hypercapnia is effective in a shallow-water crab, but ineffective in a deep-sea crab Marine Ecology Progress Series 334:1-9
Parker LM, Ross PM, O'Connor WA, Pörtner HO, Scanes E, Wright JM (2013) Predicting the response of molluscs to the impact of ocean acidification Biology 2:651-692

Parmenter RR (1980) Effects of Food Availability and Water Temperature on the Feeding Ecology of Pond Sliders (Chrysemys s. scripta) Copeia 1980:503-514 doi:10.2307/1444528
Parmesan C (2006) Ecological and Evolutionary Responses to Recent Climate Change Annual Review of Ecology, Evolution, and Systematics 37:637-669
Patton WK, Patton RJ, Barnes A (1985) On the biology of Gnathophylloides mineri, a shrimp inhabiting the sea urchin Tripneustes ventricosus Journal of Crustacean Biology 5:616-626
Pearce CM, Daggett TL, Robinson SM (2004) Effect of urchin size and diet on gonad yield and quality in the green sea urchin (Strongylocentrotus droebachiensis) Aquaculture 233:337-367
Pearce CM, Scheibling RE (1991) Effect of macroalgae, microbial films, and conspecifics on the induction of metamorphosis of the green sea urchin Strongylocentrotus droebachiensis (Mu¨ ller) Journal of Experimental Marine Biology and Ecology 147:147-162
Pearse J (1970) Reproductive periodicities of Indo-Pacific invertebrates in the Gulf of Suez. III. The echinoid Diadema setosum (Leske) Bulletin of Marine Science 20:697-720
Pearse J, Hines AH (1987) Long-term population dynamics of sea urchins in a central California kelp forest: rare recruitment and rapid decline Marine Ecology Progress Series:275-283
Pearse J, Phillips B (1968) Continuous reproduction in the Indo-Pacific sea urchin <I>Echinometra mathaei</I> at Rottnest Island, Western Australia Marine and Freshwater Research 19:161-172 doi:http://dx.doi.org/10.1071/MF9680161
Pearse JS (1969) Reproductive periodicities of Indo-Pacific invertebrates in the Gulf of Suez. II. The echinoid Echinometra mathaei (de Blainville) Bulletin of Marine Science 19:580-613
Pearse JS, Cameron RA (1991) Echinodermata: echinoidea
Pearse JS, Pearse VB, Davis KK (1986) Photoperiodic regulation of gametogenesis and growth in the sea urchin Strongylocentrotus purpuratus Journal of Experimental Zoology 237:107-118
Pecorino D, Lamare MD, Barker MF (2012) Growth, morphometrics and size structure of the Diadematidae sea urchin Centrostephanus rodgersii in northern New Zealand Marine and Freshwater Research 63:624-634
Pecorino D, Lamare MD, Barker MF (2013a) Reproduction of the Diadematidae sea urchin Centrostephanus rodgersii in a recently colonized area of northern New Zealand Marine Biology Research 9:157-168
Pecorino D, Lamare MD, Barker MF, Byrne M (2013b) How does embryonic and larval thermal tolerance contribute to the distribution of the sea urchin Centrostephanus rodgersii (Diadematidae) in New Zealand? Journal of Experimental Marine Biology and Ecology 445:120-128
Pederson HG, Johnson CR (2006) Predation of the sea urchin Heliocidaris erythrogramma by rock lobsters (Jasus edwardsii) in no-take marine reserves Journal of Experimental Marine Biology and Ecology 336:120-134 doi:http://dx.doi.org/10.1016/j.jembe.2006.04.010
Perkins NR, Hill NA, Foster SD, Barrett NS (2015) Altered niche of an ecologically significant urchin species, Centrostephanus rodgersii, in its extended range revealed using an Autonomous Underwater Vehicle Estuarine, Coastal and Shelf Science 155:56-65 doi:https://doi.org/10.1016/j.ecss.2015.01.014
Perreault M-C, Borgeaud IA, Gaymer CF (2014) Impact of grazing by the sea urchin Tetrapygus niger on the kelp Lessonia trabeculata in Northern Chile Journal of Experimental Marine Biology and Ecology 453:22-27
Perry AL, Low PJ, Ellis JR, Reynolds JD (2005) Climate Change and Distribution Shifts in Marine Fishes Science 308:1912-1915 doi:10.1126/science.1111322

Perry CT, Kench PS, O'Leary M, Morgan K, Januchowski-Hartley F (2015) Linking reef ecology to island building: Parrotfish identified as major producers of island-building sediment in the Maldives Geology 43:503-506
Peyrot-Clausade M, Chabanet P, Conand C, Fontaine M, Letourneur Y, Harmelin-Vivien M (2000) Sea urchin and fish bioerosion on La Reunion and Moorea reefs Bulletin of Marine Science 66:477-485
Phillips DL et al. (2014) Best practices for use of stable isotope mixing models in food-web studies Canadian Journal of Zoology 92:823-835
Phillips K et al. (2010) Effect of manufactured diets on the yield, biochemical composition and sensory quality of Evechinus chloroticus sea urchin gonads Aquaculture 308:49-59
Poloczanska ES et al. (2013) Global imprint of climate change on marine life Nature Climate Change 3:919-925
Pompanon F, Deagle BE, Symondson WOC, Brown DS, Jarman SN, Taberlet P (2012) Who is eating what: diet assessment using next generation sequencing Molecular Ecology 21:1931-1950 doi:10.1111/j.1365-294X.2011.05403.x
Poore AG, Graba-Landry A, Favret M, Brennand HS, Byrne M, Dworjanyn SA (2013) Direct and indirect effects of ocean acidification and warming on a marine plant–herbivore interaction Oecologia 173:1113-1124
Pörtner HO, Langenbuch M, Michaelidis B (2005) Synergistic effects of temperature extremes, hypoxia, and increases in CO2 on marine animals: From Earth history to global change Journal of Geophysical Research: Oceans 110
Qiu J-W, Lau DC, Cheang C-c, Chow W-k (2014) Community-level destruction of hard corals by the sea urchin Diadema setosum Marine pollution bulletin 85:783-788
Quinn JF, Wing SR, Botsford LW (1993) Harvest refugia in marine invertebrate fisheries: models and applications to the red sea urchin, Strongylocentrotus franciscanus American Zoologist 33:537-550
Randall JE, Schroeder R, Starck W (1964) Notes on the biology of the echinoid Diadema antillarum Caribb J Sci 4:421-433
Rasher DB, Engel S, Bonito V, Fraser GJ, Montoya JP, Hay ME (2012) Effects of herbivory, nutrients, and reef protection on algal proliferation and coral growth on a tropical reef Oecologia 169:187-198
Rasher DB, Hay ME (2010) Chemically rich seaweeds poison corals when not controlled by herbivores Proceedings of the National Academy of Sciences 107:9683-9688
Reaka-Kudla M, Feingold J, Glynn W (1996) Experimental studies of rapid bioerosion of coral reefs in the Galapagos Islands Coral Reefs 15:101-107
Richards Z et al. (2016) Marine Biodiversity in Temperate Western Australia: Multi-Taxon Surveys of Minden and Roe Reefs Diversity 8:7
Ridgway K (2007) Long-term trend and decadal variability of the southward penetration of the East Australian Current Geophysical Research Letters 34
Riegl B (2003) Climate change and coral reefs: different effects in two high-latitude areas (Arabian Gulf, South Africa) Coral Reefs 22:433-446
Rodríguez-Barreras R, Cuevas E, Cabanillas-Terán N, Sabat AM (2015a) Potential omnivory in the sea urchin Diadema antillarum? Regional Studies in Marine Science 2:11-18
Rodríguez-Barreras R, Durán A, Lopéz-Morell J, Sabat AM (2015b) Effect of fish removal on the abundance and size structure of the sea urchin Diadema antillarum: A field experiment Marine Biology Research 11:1100-1107 doi:10.1080/17451000.2015.1064140
Roff G, Mumby PJ (2012) Global disparity in the resilience of coral reefs Trends in Ecology & Evolution 27:404-413
Rose C et al. (1999) Overgrazing of a large seagrass bed by the sea urchin Lytechinus variegatus in Outer Florida Bay Marine Ecology Progress Series 190:211-222
Ruess L, Chamberlain PM (2010) The fat that matters: Soil food web analysis using fatty acids and their carbon stable isotope signature Soil Biology and Biochemistry 42:1898-1910 doi:http://dx.doi.org/10.1016/j.soilbio.2010.07.020

Sabine CL, Feely RA (2007) The oceanic sink for carbon dioxide Greenhouse gas sinks 31
Sabine CL et al. (2004) The oceanic sink for anthropogenic CO2 science 305:367-371
Sala E, Zabala M (1996) Fish predation and the structure of the sea urchin Paracentrotus lividus populations in the NW Mediterranean Marine Ecology Progress Series:71-81
Sammarco PW (1982) Echinoid grazing as a structuring force in coral communities: whole reef manipulations Journal of Experimental Marine Biology and Ecology 61:31-55
Sánchez-España AI, Martínez-Pita I, García FJ (2004) Gonadal growth and reproduction in the commercial sea urchin Paracentrotus lividus (Lamarck, 1816) (Echinodermata: Echinoidea) from southern Spain Hydrobiologia 519:61-72 doi:10.1023/B:HYDR.0000026485.40173.02
Sano M, Shimizu M, Nose Y (1987) Long-term effects of destruction of hermatypic corals by Acanthaster planci infestation on reef fish communities at Iriomote Island, Japan Mar Ecol Prog Ser 37:191-199
Scheibling R (1986) Increased macroalgal abundance following mass mortalities of sea urchins (Strongylocentrotus droebachiensis) along the Atlantic coast of Nova Scotia Oecologia 68:186-198
Scheibling RE, Hennigar AW, Balch T (1999) Destructive grazing, epiphytism, and disease: the dynamics of sea urchin-kelp interactions in Nova Scotia Canadian Journal of Fisheries and Aquatic Sciences 56:2300-2314
Scheibling RE, Lauzon-Guay J-S (2010) Killer storms: North Atlantic hurricanes and disease outbreaks in sea urchins Limnology and Oceanography 55:2331-2338
Scheibling RE, Mladenov PV (1987) The decline of the sea urchin, Tripneustes ventricosus, fishery of Barbados: a survey of fishermen and consumers Marine fisheries review 49:62-69
Scheibling RE, Stephenson R (1984) Mass mortality of Strongylocentrotus droebachiensis (Echinodermata: Echinoidea) off Nova Scotia, Canada Marine Biology 78:153-164
Schlegel P, Havenhand JN, Gillings MR, Williamson JE (2012) Individual variability in reproductive success determines winners and losers under ocean acidification: a case study with sea urchins PloS one 7:e53118
Seymour S, Paul NA, Dworjanyn SA, de Nys R (2013) Feeding preference and performance in the tropical sea urchin Tripneustes gratilla Aquaculture 400:6-13
Shears NT, Babcock RC (2002) Marine reserves demonstrate top-down control of community structure on temperate reefs Oecologia 132:131-142
Sheppard-Brennand H, Dworjanyn SA, Poore AGB (2016) Global patterns in the effects of predator declines on sea urchins Ecography:n/a-n/a doi:10.1111/ecog.02380
Shirayama Y, Thornton H (2005) Effect of increased atmospheric CO2 on shallow water marine benthos Journal of Geophysical Research: Oceans 110:C09S08 doi:10.1029/2004JC002618
Shpigel M, McBride SC, Marciano S, Lupatsch I (2004) The effect of photoperiod and temperature on the reproduction of European sea urchin Paracentrotus lividus Aquaculture 232:343-355
Shpigel M, McBride SC, Marciano S, Ron S, Ben-Amotz A (2005) Improving gonad colour and somatic index in the European sea urchin Paracentrotus lividus Aquaculture 245:101-109
Silbiger NJ, Guadayol Ò, Thomas FI, Donahue MJ (2014) Reefs shift from net accretion to net erosion along a natural environmental gradient Marine Ecology Progress Series 515:33-44
Simoncini M, Miller RJ (2007) Feeding preference of Strongylocentrotus droebachiensis (Echinoidea) for a dominant native ascidian, Aplidium glabrum, relative to the invasive ascidian Botrylloides violaceus Journal of experimental marine biology and ecology 342:93-98

Solomon S, Plattner G-K, Knutti R, Friedlingstein P (2009) Irreversible climate change due to carbon dioxide emissions Proceedings of the national academy of sciences:pnas. 0812721106
Steinacher M, Joos F, Frolicher T, Plattner G-K, Doney SC (2009) Imminent ocean acidification in the Arctic projected with the NCAR global coupled carbon cycle-climate model
Steneck RS (2013) Sea urchins as drivers of shallow benthic marine community structure Sea Urchins: Biology and Ecology:195-207
Steneck RS, Graham MH, Bourque BJ, Corbett D, Erlandson JM, Estes JA, Tegner MJ (2002) Kelp forest ecosystems: biodiversity, stability, resilience and future Environmental conservation 29:436-459
Stevenson A, Rocha C (2013) Evidence for the bioerosion of deep-water corals by echinoids in the Northeast Atlantic Deep Sea Research Part I: Oceanographic Research Papers 71:73-78
Stewart NL, Konar B (2012) Kelp forests versus urchin barrens: alternate stable states and their effect on sea otter prey quality in the Aleutian Islands Journal of Marine Biology 2012
Stimson J, Cunha T, Philippoff J (2007) Food preferences and related behavior of the browsing sea urchin Tripneustes gratilla (Linnaeus) and its potential for use as a biological control agent Marine Biology 151:1761-1772
Stumpp M, Hu M, Casties I, Saborowski R, Bleich M, Melzner F, Dupont S (2013) Digestion in sea urchin larvae impaired under ocean acidification Nature climate change 3:1044-1049
Stumpp M et al. (2012) Acidified seawater impacts sea urchin larvae pH regulatory systems relevant for calcification Proceedings of the National Academy of Sciences 109:18192-18197
Sunday JM, Calosi P, Dupont S, Munday PL, Stillman JH, Reusch TB (2014) Evolution in an acidifying ocean Trends in Ecology & Evolution 29:117-125
Tegner M, Dayton P (1981) Population structure, recruitment and mortality of two sea urchins (Strongylocentrotus franciscanus and S. purpuratus) in a kelp forest Mar Ecol Prog Ser 5:68
Tegner MJ, Levin LA (1983) Spiny lobsters and sea urchins: Analysis of a predator-prey interaction Journal of Experimental Marine Biology and Ecology 73:125-150 doi:https://doi.org/10.1016/0022-0981(83)90079-5
Thompson R (1983) The relationship between food ration and reproductive effort in the green sea urchin, Strongylocentrotus droebachiensis Oecologia 56:50-57
Thomson D, Babcock R, Vanderklift M, Symonds G, Gunson J (2012) Evidence for persistent patch structure on temperate reefs and multiple hypotheses for their creation and maintenance Estuarine, Coastal and Shelf Science 96:105-113
Thomson DP, Frisch AJ (2010) Extraordinarily high coral cover on a nearshore, high-latitude reef in south-west Australia Coral Reefs 29:923-927 doi:10.1007/s00338-010-0650-1
Thurber RV et al. (2012) Macroalgae decrease growth and alter microbial community structure of the reef-building coral, Porites astreoides PLoS One 7:e44246
Tribollet A, Decherf G, Hutchings PA, Peyrot-Clausade M (2002) Large-scale spatial variability in bioerosion of experimental coral substrates on the Great Barrier Reef (Australia): Importance of microborers Coral Reefs 21:424-432
Tribollet A, Golubic S (2011) Reef bioerosion: agents and processes. In: Coral reefs: an ecosystem in transition. Springer, pp 435-449
Tsuda F, Hoshikawa H, Agatsuma Y, Taniguchi K (2006) Gonad production of the sea urchin Strongylocentrotus nudus in relation to algal vegetation in shallow waters along the Sea of Japan, southwestern Hokkaido, Japan Sci Rep Hokkaido Fish Exp Stn 71:63-68
Tuason A, Gomez ED The reproductive biology of Tripneustes gratilla Linnaeus (Echinodermata: Echinoidea), with some notes on Diadema setosum Leske. In: Proceedings of the International Symposium of Marine Biogeography and Evolution in the Southern Hemisphere. Auckland, New Zealand, 1979. pp 707-716

Tuya F, Boyra A, Sanchez-Jerez P, Barbera C, Haroun RJ (2004) Relationships between rocky-reef fish assemblages, the sea urchin Diadema antillarum and macroalgae throughout the Canarian Archipelago Marine Ecology Progress Series 278:157-169
Uthicke S, Liddy M, Nguyen H, Byrne M (2014) Interactive effects of near-future temperature increase and ocean acidification on physiology and gonad development in adult Pacific sea urchin, Echinometra sp. A Coral Reefs 33:831-845
Uthicke S, Schaffelke B, Byrne M (2009) A boom–bust phylum? Ecological and evolutionary consequences of density variations in echinoderms Ecological Monographs 79:3-24
Vaitilingon D, Rasolofonirina R, Jangoux M (2005) Reproductive Cycle of Edible Echinoderms from the Southwestern Indian Ocean I. Tripneustes gratilla L.(Echinoidea, Echinodermatata) Western Indian Ocean journal of marine science 4:47-60
Vaïtilingon D, Rasolofonirina R, Jangoux M (2003) Feeding preferences, seasonal gut repletion indices, and diel feeding patterns of the sea urchin Tripneustes gratilla (Echinodermata: Echinoidea) on a coastal habitat off Toliara (Madagascar) Marine biology 143:451-458
Valentine JP, Johnson CR (2005) Persistence of sea urchin (Heliocidaris erythrogramma) barrens on the east coast of Tasmania: inhibition of macroalgal recovery in the absence of high densities of sea urchins Botanica Marina 48:106-115
Valentini A, Pompanon F, Taberlet P (2009) DNA barcoding for ecologists Trends Ecol Evol 24:110-117 doi:10.1016/j.tree.2008.09.011
Vanderklift M, Lavery P, Waddington K (2009) Intensity of herbivory on kelp by fish and sea urchins differs between inshore and offshore reefs
Vanderklift MA, Kendrick GA (2004) Variation in abundances of herbivorous invertebrates in temperate subtidal rocky reef habitats Marine and Freshwater Research 55:93-103
Vanderklift MA, Kendrick GA (2005) Contrasting influence of sea urchins on attached and drift macroalgae Marine Ecology Progress Series 299:101-110
Vanderklift MA, Kendrick GA, Smit AJ (2006) Differences in trophic position among sympatric sea urchin species Estuarine, Coastal and Shelf Science 66:291-297
Vanderklift MA, Ponsard S (2003) Sources of variation in consumer-diet δ15N enrichment: a meta-analysis Oecologia 136:169-182
Vanderklift MA, Wernberg T (2008) Detached kelps from distant sources are a food subsidy for sea urchins Oecologia 157:327-335
Vergés A et al. The tropicalization of temperate marine ecosystems: climate-mediated changes in herbivory and community phase shifts. In: Proc. R. Soc. B, 2014. vol 1789. The Royal Society, p 20140846
Veron JEN (1995) Corals in space and time: the biogeography and evolution of the Scleractinia. Cornell University Press,
Veron JEN et al. (2009) The coral reef crisis: The critical importance of <350 ppm CO2 Marine Pollution Bulletin 58:1428-1436 doi:http://dx.doi.org/10.1016/j.marpolbul.2009.09.009
Veron JJ, Marsh L (1988) Hermatypic corals of Western Australia. Records and annotated species list. Supplement Records of the Western Australian Museum-pages: 29: 1-136
Wagner SC (2012) Keystone species Nature Education Knowledge 3:51
Walker MM (1982) Reproductive periodicity in Evechinus chloroticus in the Hauraki Gulf New Zealand Journal of Marine and Freshwater Research 16:19-25 doi:10.1080/00288330.1982.9515944
Wallner-Hahn S, de la Torre-Castro M, Eklöf JS, Gullström M, Muthiga NA, Uku J (2015) Cascade effects and sea-urchin overgrazing: An analysis of drivers behind the exploitation of sea urchin predators for management improvement Ocean & Coastal Management 107:16-27
Wangensteen OS, Turon X, García-Cisneros A, Recasens M, Romero J, Palacín C (2011) A wolf in sheep's clothing: carnivory in dominant sea urchins in the Mediterranean Marine Ecology Progress Series 441:117-128

Wassmann P, Duarte CM, Agusti S, Sejr MK (2011) Footprints of climate change in the Arctic marine ecosystem Global change biology 17:1235-1249
Webster FJ, Babcock RC, Van Keulen M, Loneragan NR (2015) Macroalgae inhibits larval settlement and increases recruit mortality at Ningaloo Reef, Western Australia PloS one 10:e0124162
Whalan S, Webster NS, Negri AP (2012) Crustose Coralline Algae and a Cnidarian Neuropeptide Trigger Larval Settlement in Two Coral Reef Sponges PLOS ONE 7:e30386 doi:10.1371/journal.pone.0030386
Wilmers CC, Estes JA, Edwards M, Laidre KL, Konar B (2012) Do trophic cascades affect the storage and flux of atmospheric carbon? An analysis of sea otters and kelp forests Frontiers in Ecology and the Environment 10:409-415
Wing SR (2009) Decadal-scale dynamics of sea urchin population networks in Fiordland, New Zealand are driven by juxtaposition of larval transport against benthic productivity gradients Marine Ecology Progress Series 378:125-134
Wisshak M, Schönberg CH, Form A, Freiwald A (2012) Ocean acidification accelerates reef bioerosion PloS one 7:e45124
Wolf-Gladrow DA, Riebesell ULF, Burkhardt S, Bijma J (1999) Direct effects of CO2 concentration on growth and isotopic composition of marine plankton Tellus B 51:461-476 doi:10.1034/j.1600-0889.1999.00023.x
Worm B, Lotze HK, Hillebrand H, Sommer U (2002) Consumer versus resource control of species diversity and ecosystem functioning Nature 417:848-851
Wright JT, Dworjanyn SA, Rogers CN, Steinberg PD, Williamson JE, Poore AG (2005) Density-dependent sea urchin grazing: differential removal of species, changes in community composition and alternative community states Marine Ecology Progress Series 298:143-156
Young M, Bellwood D (2012) Fish predation on sea urchins on the Great Barrier Reef Coral Reefs 31:731-738
Zanden MJV, Rasmussen JB (2001) Variation in δ15N and δ13C Trophic Fractionation: Implications for Aquatic Food Web Studies Limnology and Oceanography 46:2061-2066 doi:10.2307/3069075
Zeebe RE, Wolf-Gladrow DA (2001) CO2 in seawater: equilibrium, kinetics, isotopes. vol 65. Gulf Professional Publishing,
Zieman J, Macko S, Mills A (1984) Role of seagrasses and mangroves in estuarine food webs: temporal and spatial changes in stable isotope composition and amino acid content during decomposition Bulletin of Marine Science 35:380-392